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TITLE: Identification and Targeting of Tyrosine Kinase Activity in Prostate Cancer Initiation, Progression, and Metastasis

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14. ABSTRACT

In metastatic castration resistant prostate cancer (CRPC) activation of kinase pathways may provide resistance to androgen withdrawal in the absence of activating mutations. To gain a better understanding of kinase activation patterns in metastatic CRPC, we utilized phospho-tyrosine peptide enrichment and quantitative mass spectrometry to identify druggable targets in metastatic CRPC patients obtained at rapid autopsy. Evaluation of these rare metastatic CRPC samples for tyrosine phosphorylation and kinases revealed activated SRC, EGFR, RET, ALK, and MAPK1/3 and other targets. Importantly, these kinase activation patterns exhibited intrapatient similarity and interpatient heterogeneity implying clonal origins of these lesions. Phosphoproteomic analyses and identification of kinase activation states in metastatic CRPC patients have allowed for the prioritization of kinases for further clinical evaluation.

15. SUBJECT TERMS

Prostate cancer, metastasis, tyrosine kinase, signaling, phosphoproteomics, clinical, human

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INTRODUCTION

Prostate cancer is the most commonly diagnosed and second leading cause of cancer-related death in American men (1). Death is usually a result of the spread of prostate cells to other sites in the body (metastasis) (2). Despite this high rate of mortality, little is understood about prostate cancer metastasis and in particular how to effectively treat this disease. Tyrosine kinase activity has been shown to play a role in prostate cancer, but targeting these same molecules clinically has not been very successful (3). The purpose of this grant was to provide important insights into which tyrosine kinases are crucial for advanced prostate cancer and, more importantly, help to elucidate better treatment strategies for this disease. The utilization of phosphopeptide enrichment and mass spectrometry will enable identification of these new targets in prostate cancer which may be investigated further for clinical benefit.

BODY

Over the last year, my main focus was on Aim 3 using human clinical prostate tissue for evaluation of tyrosine kinases and significant progress was made including a first author publication (Drake et al., *PNAS* In press, attached in appendices). Below are the current updates to my proposed work.

Aim 3: Identify the activated tyrosine kinases in advanced prostate cancer in man.

Parts a and b are completed. We have initiated Part c and currently developing a library of tyrosine kinases for functional validation.

a. We have completed Part a. We initiated a collaboration with the lab of Ken Pienta (University of Michigan, now at Johns Hopkins University) to obtain metastatic castration resistant prostate cancer (CRPC) tissue through their Warm Autopsy program (5). Collection of metastatic CRPC tissue is very difficult to obtain but we were able to acquire over 40 tissue samples, including enough material to evaluate 16 samples for phosphoproteomics (Figure 1).

b. We ran 16 metastatic CRPC samples, 6 benign and cancer matched treatment naïve prostates (controls), vanadate treated 22Rv1, C4-2, and DU145 prostate cancer cell lines, and cell line-derived xenograft metastatic tumors described in Aim 2b (Figure 2). This initial run identified 3 unique clusters of phosphopeptides, including a separate cluster for the cell line-derived xenografts, treatment naïve prostate cancers, and metastatic CRPC samples (Figure 2A, B). Further, we were able to show patient-specific patterns of tyrosine kinase activity (Figure 2C), including similar patterns from multiple metastatic lesions derived from the same patient (Figure 3, 4). Principal component analyses (Figure 5) and statistical evaluation of this finding was confirmed (Figure 6A, B).

c. This Part has been initiated and we are using Gateway Recombination Technology to systematically clone our identified tyrosine kinases into lentiviral vectors for functional validation (Figure 7). Once we achieve this, we will be our experiments evaluating the role of these activated kinases in prostate cancer.

KEY RESEARCH ACCOMPLISHMENTS

- Tyrosine kinase evaluation of a significant cohort of rare metastatic CRPC tissues using unbiased phosphoproteomic analyses
- Identified that cell line-derived xenografts express different kinase patterns when compared to primary tissues (including treatment naïve prostate cancer and metastatic CRPC)
- Treatment naïve prostate cancer tissues express different kinase activities from metastatic CRPC tissues
- Identified kinase activities which revealed intra-patient similarities and inter-patient differences
- Phosphoproteomic analyses and kinase:substrate relationship analysis of the phosphopeptides revealed SRC, AKT, MAPK1/3, ALK, and RET tyrosine kinase activation. All these kinases have the potential to be targetable using FDA approved tyrosine kinase inhibitors.

REPORTABLE OUTCOMES

- Published a first-author manuscript in *PNAS* (In Press, see appendices)
- Presented research findings at the UCLA Stem Cell Conference (February 2013). (see appendices)

- Presented research findings at the Prostate Cancer Foundation Prouts Neck Meeting on Prostate Cancer in Lake Tahoe, NV (June 2013). (see appendices)
- Presented research findings at the Gordon Research Conference on Hormone Dependent Cancers in Smithfield, RI (July 2013). (see appendices)
- Data deposition from the *PNAS* manuscript: MS2 spectra for all phosphopeptides reported in this paper have been deposited in the Proteome Xchange database, http://www.proteomexchange.org/ (accession no. PXD000238).
- Filed a provisional patent "Identification of phosphopeptides in prostate cancer for therapeutic treatment." UCLA 2013-854-1, U.S. Provisional Application Serial No. 61/836,984 (see appendices).

CONCLUSION

Metastatic castration resistant prostate cancer (CRPC) remains incurable due to the lack of effective therapies. The purpose of this award was to utilize different technologies to identify new therapeutic targets in advanced prostate cancer. The need to identify new actionable targets in CRPC is crucial as we begin to examine the resistance mechanisms related to androgen withdrawal. Here, we report an unbiased quantitative phosphoproteomic approach to identify druggable kinases in rare, lethal metastatic CRPC tissues. These kinase activation patterns revealed intrapatient similarity and interpatient heterogeneity across a large panel of kinase targets. Interestingly, these kinase activities are not a result of mutation but rather pathway activation within the tumors themselves. The observation that similar kinase activities are present in most if not all anatomically disparate metastatic lesions from the same patient suggests that CRPC patients may benefit from individualized, targeted combination therapies.

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3. **Araujo JC, *et al.* (2011) Dasatinib combined with docetaxel for castration-resistant prostate cancer: Results from a phase 1-2 study. *Cancer.***
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5. **Rubin MA, *et al.* (2000) Rapid ("warm") autopsy study for procurement of metastatic prostate cancer. *Clin Cancer Res* 6(3):1038-1045.**

APPENDICES (see attached)

- Supporting Data (Figures 1-7)
- Drake et al. *PNAS* Manuscript (In press)
- Provisional Patent Application
- 2013 Stem Cell Conference Abstract
- 2013 Prostate Cancer Foundation Prouts Neck Meeting on Prostate Cancer Research Program
- 2013 Gordon Research Conference on Hormone Dependent Cancers Abstract
- 2013 Gordon Research Conference on Hormone Dependent Cancers Research Program

Supporting Data

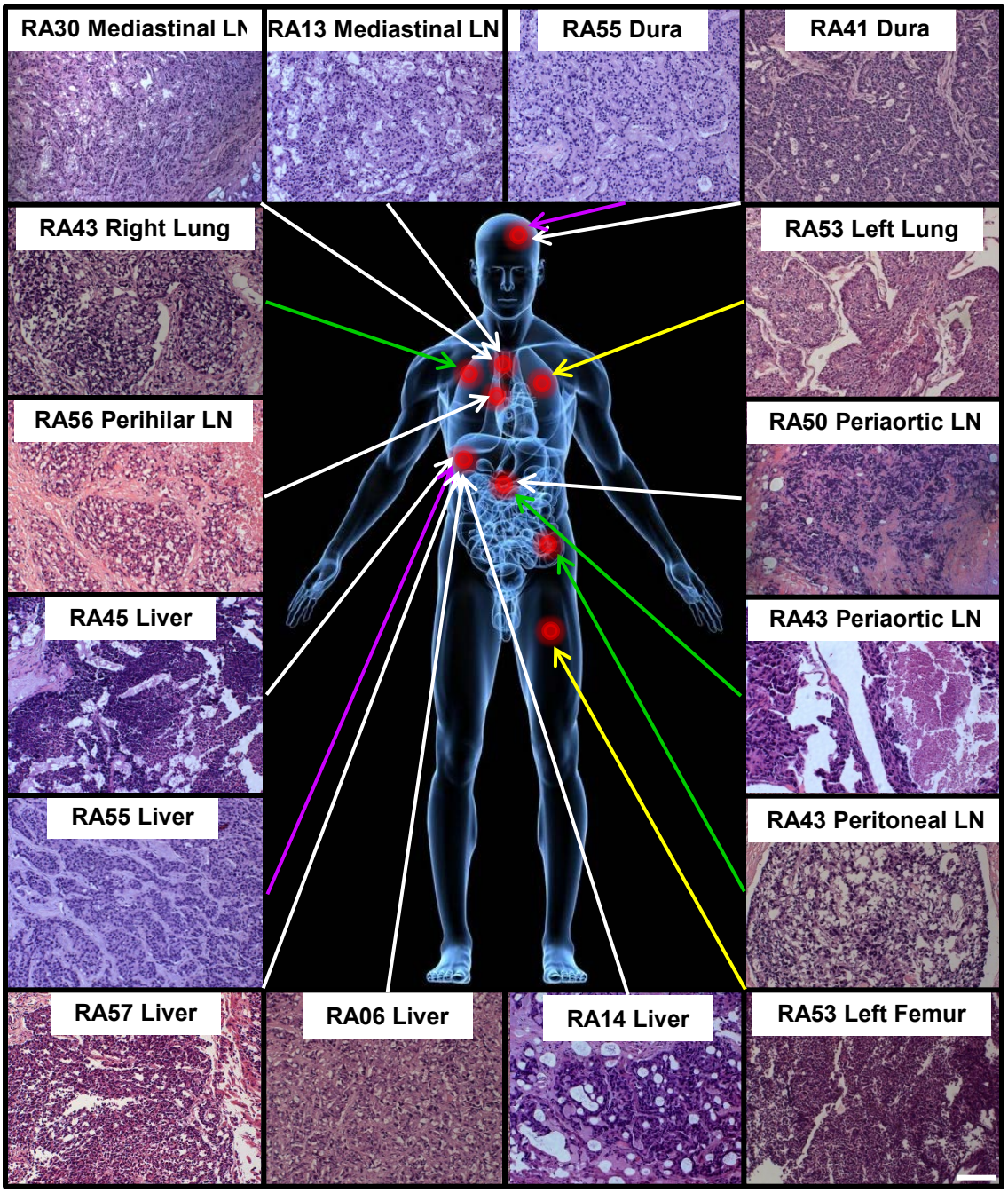

Figure 1. Anatomical location and histological characterization of metastatic castration resistant prostate cancer (CRPC) samples used for phospho-proteomics. Metastatic CRPC tissues were obtained from the Rapid Autopsy Program at The University of Michigan. 16 samples from 12 different patients are represented and prepared as previously described for phospho-proteomics (8). Red dots indicate the approximate location of the metastatic lesions analyzed. Same-colored lines represent tissues from the same patient. Patient RA53 left lung and left femur were combined due to limiting material (yellow lines). Only tissues with greater than 350 mg and 50% tumor content were evaluated. Scale bar=50 μm.

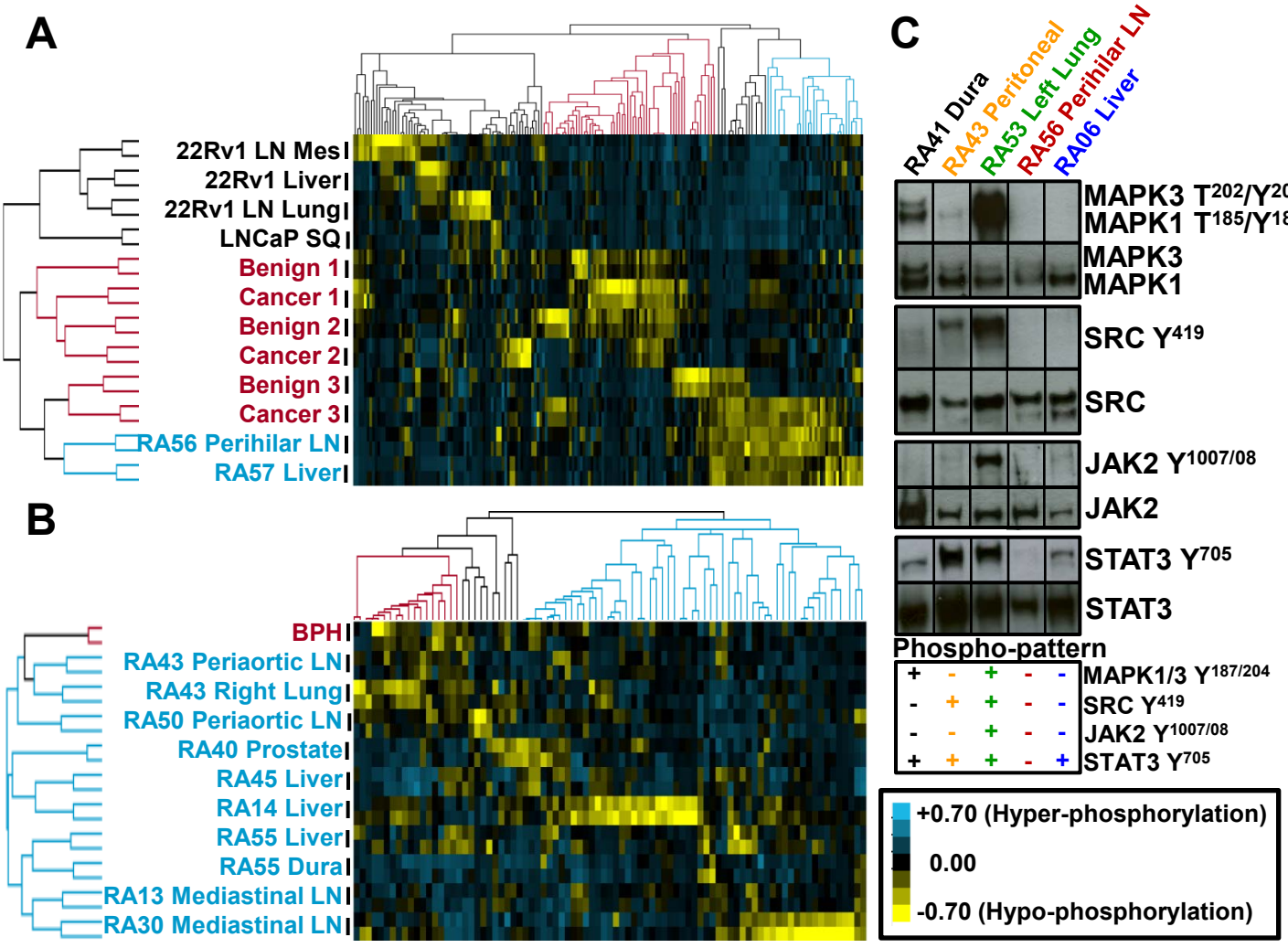

Figure 2. Phosphoproteomic analyses of cell line-derived xenografts, treatment naïve prostate cancer, and metastatic CRPC reveals distinct phospho-patterns. (A) Unsupervised hierarchical clustering of phospho-tyrosine enriched peptides separates cell line-derived xenograft tumors from primary prostate or metastatic tissue. (B) Further evaluation of a separate run of 10 metastatic CRPC lesions reveals patient-specific and metastatic site similarity of phospho-tyrosine peptide patterns. (C) Western blot validation of 4 different activated kinases identified from both phospho-proteomics and inferred kinase activities confirms the heterogeneity observed across 5 different patients as each patient exhibited a unique phospho-pattern. Western blot data was separated to highlight each individual patient but were performed on the same western blot. Yellow=hyperphosphorylation, Blue=hypophosphorylation. Intensity bar in Fig. 2B is applicable to Fig. 2A.

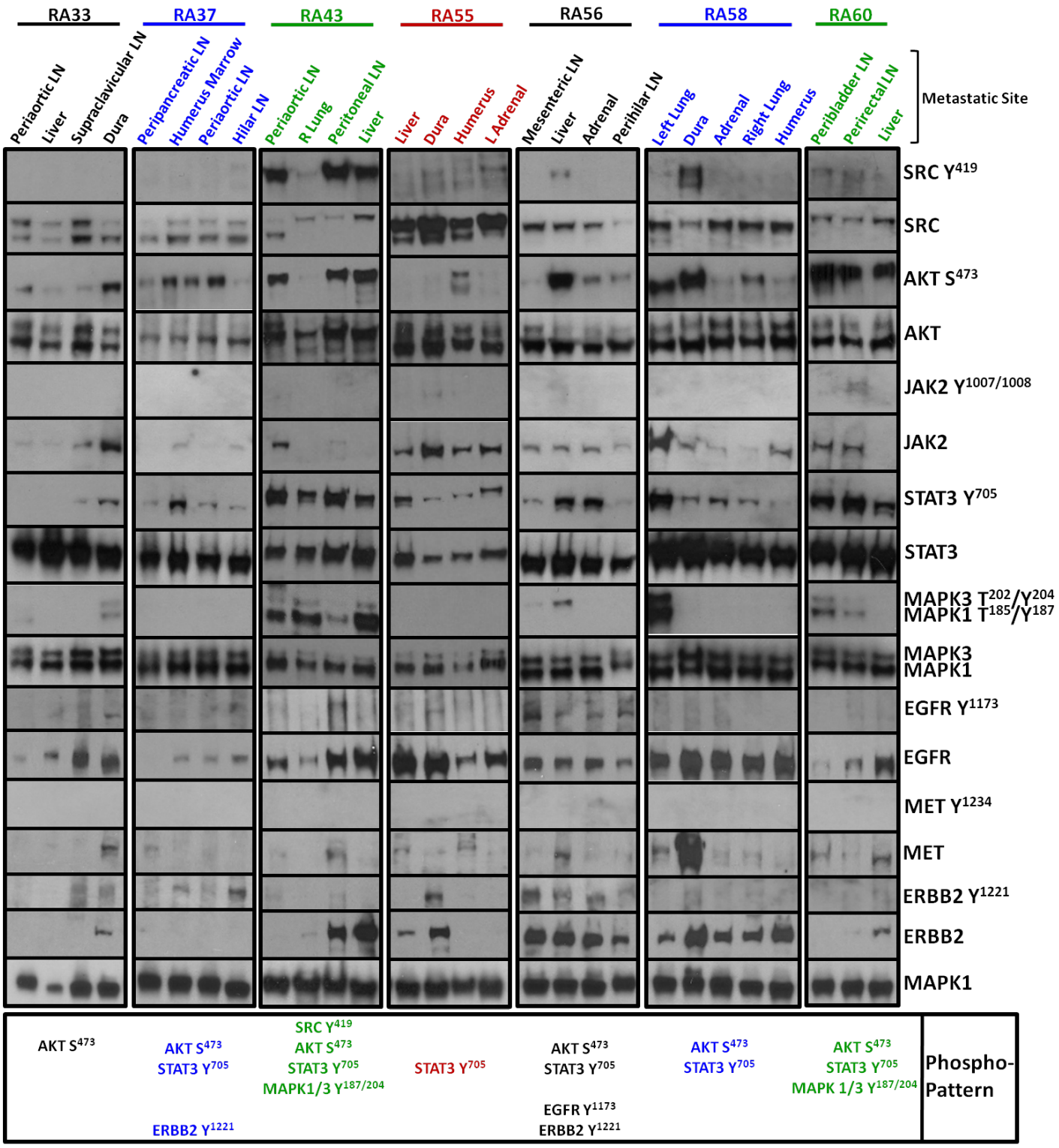

Figure 3. Related phospho-kinase and substrate expression patterns are observed within distinct anatomical metastatic lesions of the same patient. Western blot analyses from 7 different sets of patients with 3 or 4 distinct metastatic lesions were evaluated for kinase activation patterns that were identified in the phospho-proteomic data sets and kinase/substrate relationships or receptor tyrosine kinases that have been previously targeted clinically. Each patient expressed similar activated kinase patterns independent of the anatomical location of the metastatic lesions. The unique phospho-patterns are also depicted schematically below the western blot data.

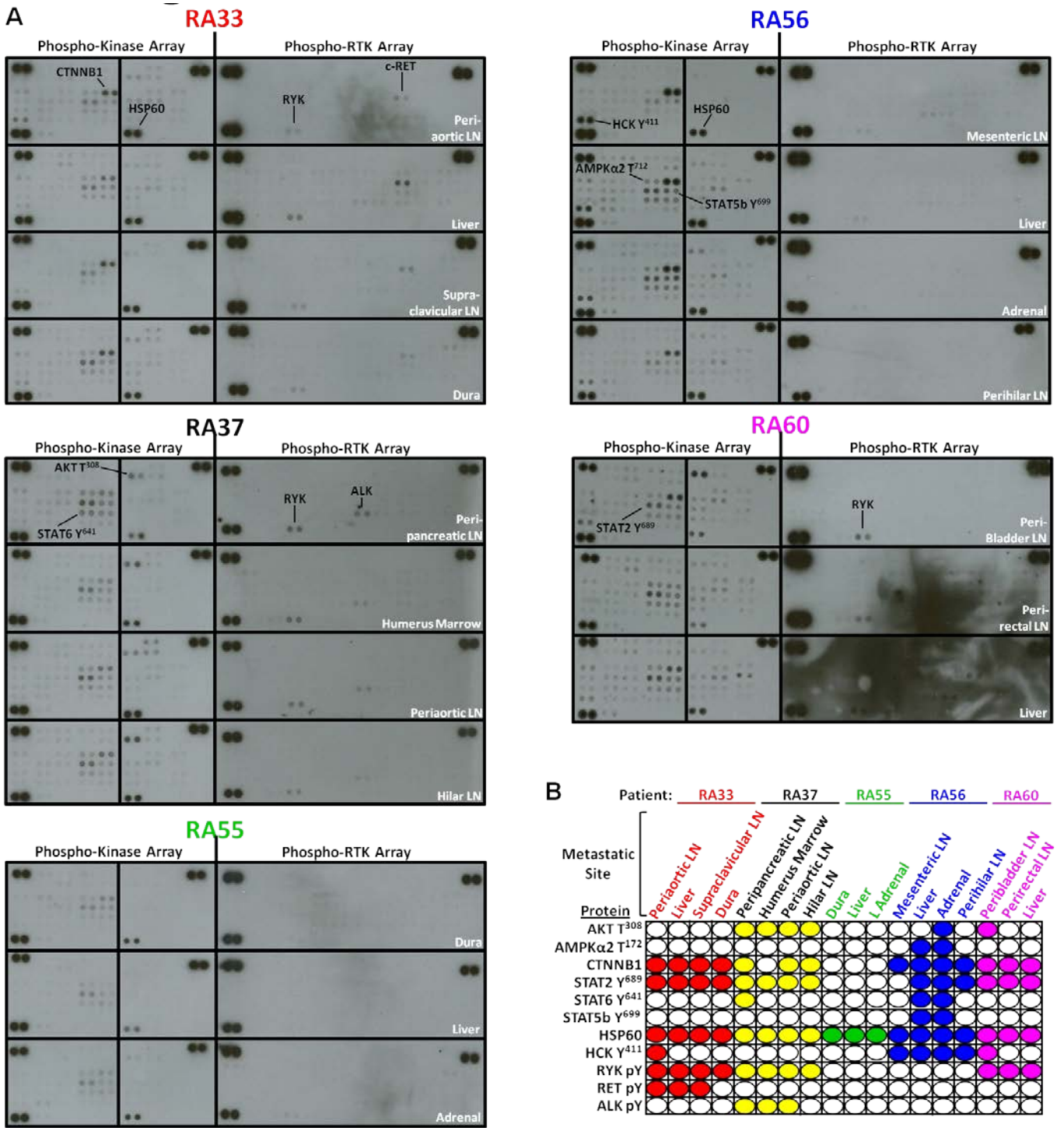

Figure 4. Large scale analyses of kinase activation patterns confirms intrapatient similarity across multiple, anatomically distinct metastases. (A) Phospho-kinase and phospho-RTK arrays were used to analyze metastatic lesions from 5 different patients from anatomically distinct metastatic lesions. (B) Unique phospho-patterns were observed for each patient and similar patterns were observed within the same patient as shown with like-colored circles. Each observable phospho- or total protein spot from the phospho-kinase and RTK arrays were used for principal component analysis (PCA).

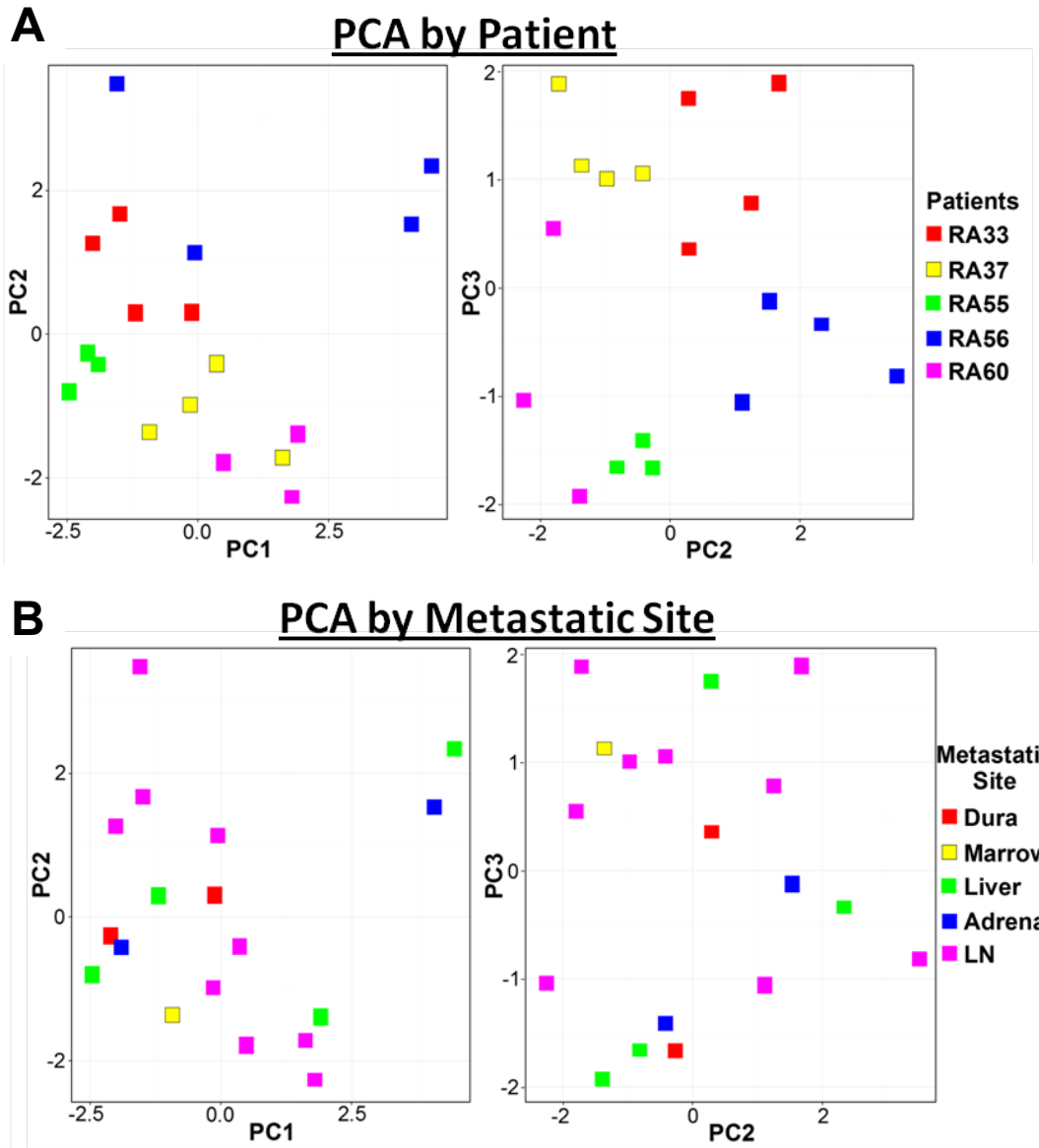

Figure 5. Principal component analysis (PCA) of kinase activation patterns confirms intrapatient similarity across multiple, anatomically distinct metastases. (A) PCA analysis of all 5 patients confirms intra-patient kinase expression similarity and interpatient dissimilarity. (B) Grouping metastatic lesions by similar anatomical site shows no significant grouping of samples. Each phospho-kinase and phospho-RTK array are spotted in duplicate and positive control spots are located in the top left, right, and bottom left of each array. The first three principal components represent 77% of the total variance. Marrow=bone marrow lesion; adrenal=adrenal gland lesions; LN=distant lymph node lesions

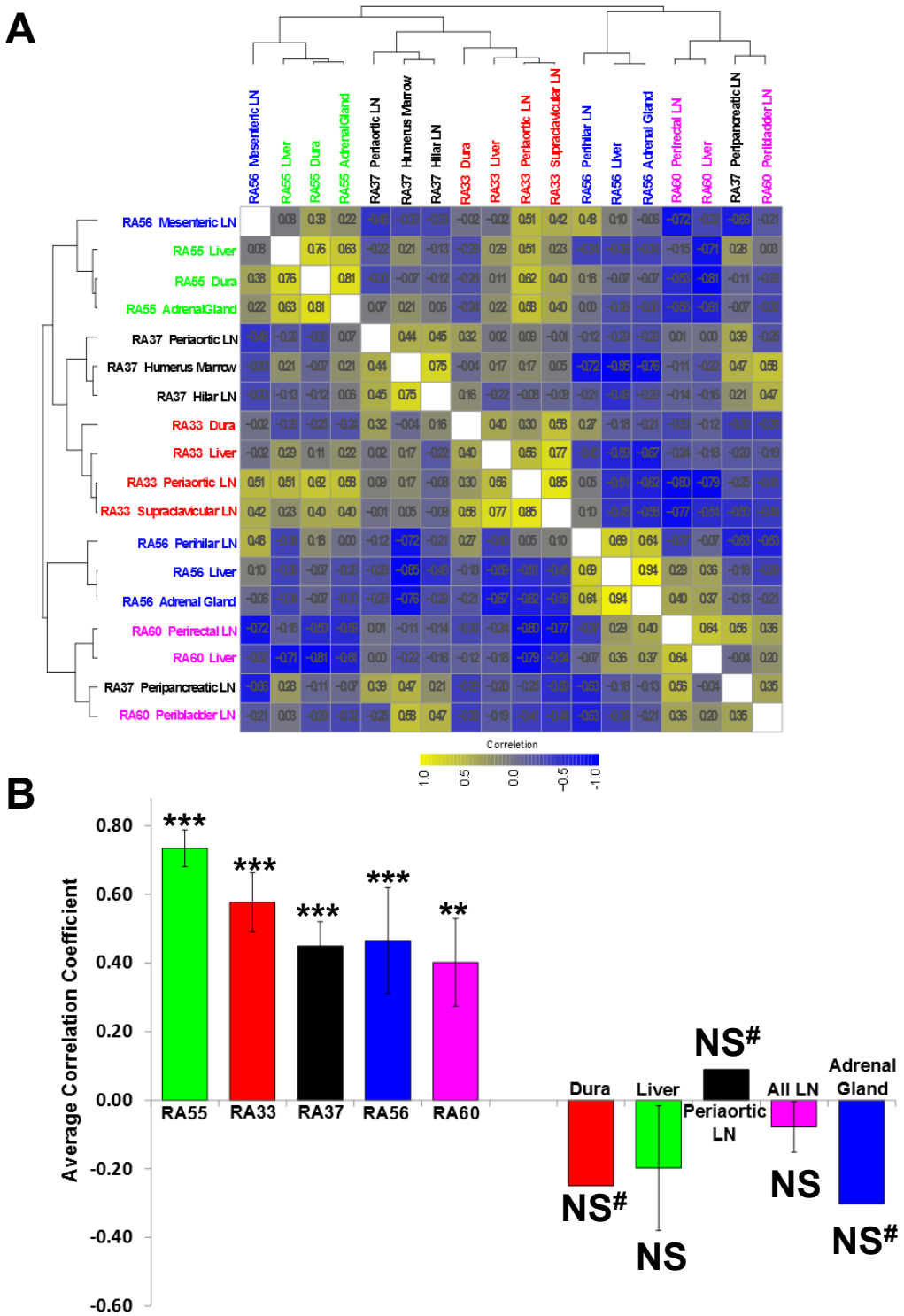

Figure 6. Phospho-kinase arrays demonstrate high levels of intrapatient but not interpatient similarity. (A) Pairwise Pearson correlation coefficients for each sample measured on the phospho-kinase and phospho-RTK arrays were calculated and then clustered. The correlation coefficients are superimposed on each color-coded square. The correlation coefficients on the diagonal were omitted for readability. (B) Pairwise correlation coefficients were averaged, and the statistical significance against the null hypothesis that the correlation was not greater than zero was calculated. Error bars are the standard error. Multiple p-values were combined using Fisher's Method. ***p<0.001, **p<0.01, NS = not significant, # = single p-value, not Fisher's combined.

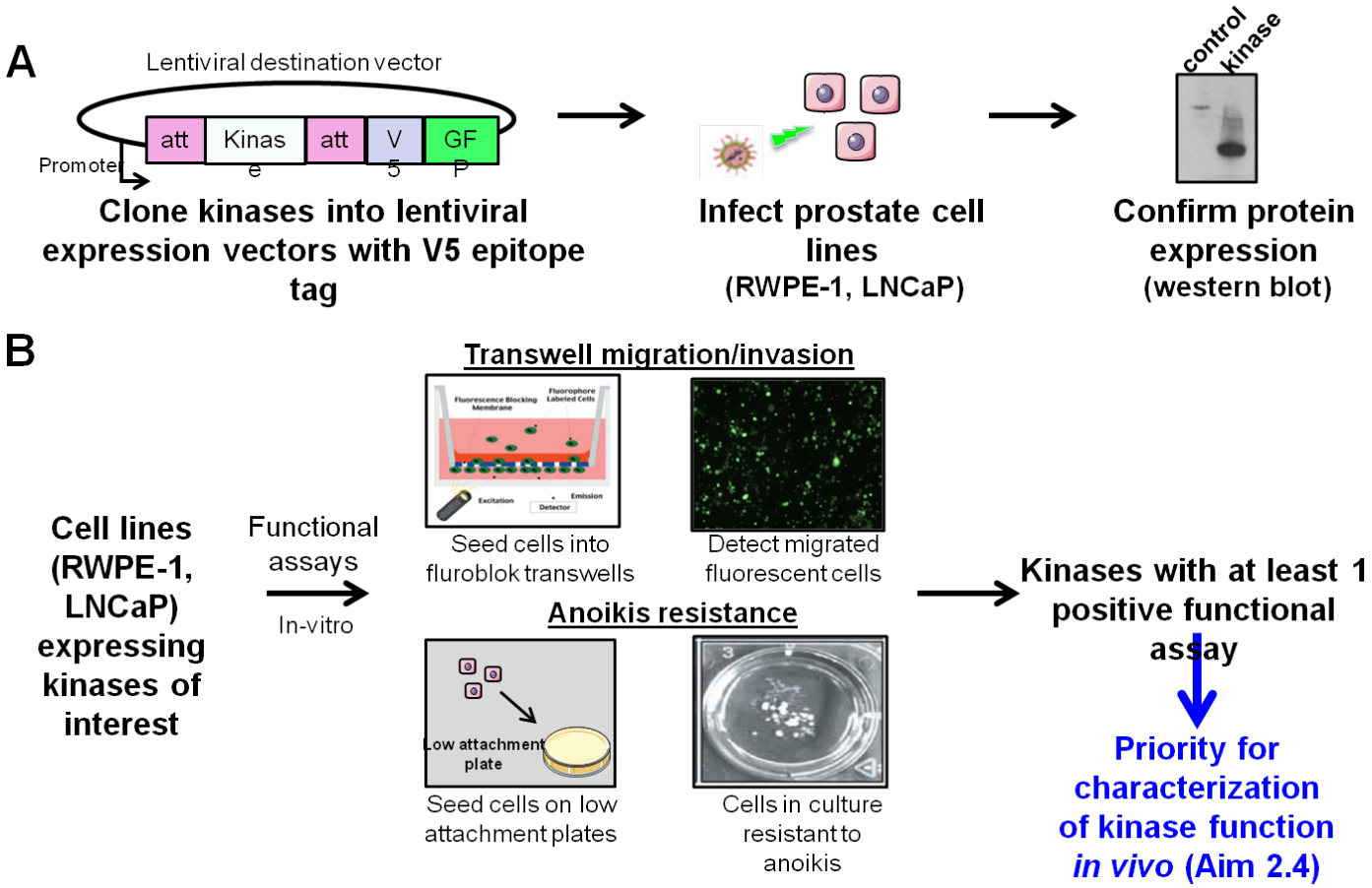

Figure 7. Assess functional significance of identified kinases *in vitro*. (A) Schematic diagram of lentiviral vector used for transducing cell lines expressing kinase of interest. These transduced cell lines will then be subjected to four different *in vitro* assays (B) to measure cell migration/invasion and resistance to anoikis. Kinases that are positive in 3/4 assays will be assessed *in vivo.*

Metastatic castration-resistant prostate cancer reveals intrapatient similarity and interpatient heterogeneity of therapeutic kinase targets

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Contributed by Owen N. Witte, October 23, 2013 (sent for review September 5, 2013)

In prostate cancer, multiple metastases from the same patient share similar copy number, mutational status, erythroblast transformation specific (ETS) rearrangements, and methylation patterns supporting their clonal origins. Whether actionable targets such as tyrosine kinases are also similarly expressed and activated in anatomically distinct metastatic lesions of the same patient is not known. We evaluated active kinases using phosphotyrosine peptide enrichment and quantitative mass spectrometry to identify druggable targets in metastatic castration-resistant prostate cancer obtained at rapid autopsy. We identified distinct phosphopeptide patterns in metastatic tissues compared with treatment-naive primary prostate tissue and prostate cancer cell line-derived xenografts. Evaluation of metastatic castration-resistant prostate cancer samples for tyrosine phosphorylation and upstream kinase targets revealed SRC, epidermal growth factor receptor (EGFR), rearranged during transfection (RET), anaplastic lymphoma kinase (ALK), and MAPK1/3 and other activities while exhibiting intrapatient similarity and interpatient heterogeneity. Phosphoproteomic analyses and identification of kinase activation states in metastatic castration-resistant prostate cancer patients have allowed for the prioritization of kinases for further clinical evaluation.

metastasis | resistance | personalized medicine | combination therapy | phosphotyrosine

Mutational and copy number analyses from epithelial tumors have identified several activating tyrosine kinase mutations and amplifications, such as epidermal growth factor receptor (EGFR) mutations in lung adenocarcinoma and erythroblastic leukemia viral oncogene homolog 2 (*ERBB2 or HER2/neu*) gene amplification in breast cancer (1). The dependence on these tyrosine kinases for tumor growth and survival has led to successful clinical treatment with tyrosine kinase inhibitors (TKIs) (2, 3). However, recent genomic analyses of prostate adenocarcinoma revealed that activating tyrosine kinase mutations or amplifications are very rare (1, 4–6).

Despite the scarcity of tyrosine kinase amplifications or activating mutations in prostate cancer, tyrosine kinase expression and activity has been shown to play an important role in disease progression. For example, coexpression of wild-type SRC tyrosine kinase and androgen receptor (AR) can synergistically drive the formation of mouse prostate adenocarcinoma (7). Evaluation of nontyrosine-kinase–initiated mouse models of prostate cancer further identified activation of the nonreceptor tyrosine kinases SRC, ABL1, and Janus kinase 2 (JAK2) (8). We also observed increased tyrosine phosphorylation in nearly 50% of castration-resistant prostate cancer (CRPC) tissues examined compared with hormone-naïve prostate cancer (8). These studies suggest that comprehensive evaluation of metastatic CRPC samples for tyrosine kinase activity may lead to the identification of new drug targets.

Studies in melanoma and breast cancer have revealed that despite heterogeneity in primary, localized disease, metastases seem to arise from a single precursor cell (9, 10). The multifocal nature of organ-confined prostate cancer poses a question as to the clonality of metastatic disease (11). Investigation into clonality in metastatic CRPC has found that tumors isolated from anatomically different lesions in the same patient bear similar copy number, mutational status, erythroblast transformation specific (ETS) rearrangements, and methylation patterns from multiple metastatic lesions supporting their clonal origins (6, 12–14). In addition, these studies found a remarkable amount of interpatient heterogeneity, suggesting that personalized medicine approaches may be necessary to efficiently target metastatic lesions. Previous observations of intrapatient similarity hold promise with regard to treatment strategies for metastatic CRPC patients by means of systematically attacking the cancer cell clone contributing to disease.

This led us to investigate whether actionable targets such as tyrosine kinases also maintain similar activation patterns across anatomically distinct metastases from the same patient. With

Significance

Metastatic castration-resistant prostate cancer (CRPC) remains incurable due to the lack of effective therapies. The need to identify new actionable targets in CRPC is crucial as we begin to examine the resistance mechanisms related to androgen withdrawal. Here, we report an unbiased quantitative phosphoproteomic approach to identify druggable kinases in metastatic CRPC. These kinase activation patterns revealed intrapatient similarity and interpatient heterogeneity across a large panel of targets. Interestingly, these kinase activities are not a result of mutation but rather pathway activation within the tumors themselves. The observation that similar kinase activities are present in most if not all anatomically disparate metastatic lesions from the same patient suggests that CRPC patients may benefit from individualized, targeted combination therapies.

Author contributions: J.M.D., N.A.G., K.J.P., T.G.G., and O.N.W. designed research; J.M.D., N.A.G., J.K.L., T.S., C.M.F., and S.S. performed research; J.M.D., N.A.G., J.K.L., C.M.F., B.T., and J.H. analyzed data; and J.M.D., N.A.G., J.K.L., and O.N.W. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

Data deposition: The MS proteomics data have been deposited in ProteomeXchange, www.proteomexchange.org (accession no. PXD000238).

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access to rare metastatic CRPC tissue from the University of Michigan's Rapid Autopsy Program (15), we evaluated global tyrosine phosphorylation patterns in lethal metastatic CRPC patients. Phosphotyrosine peptide enrichment and quantitative mass spectrometry (MS) identified diverse phosphorylation events in the metastatic tissues compared with naive primary prostate tissue and prostate cancer cell line-derived xenografts. Validation of activated kinases that were identified via either MS or kinase–substrate relationships revealed intrapatient similarity and interpatient heterogeneity across a large panel of targets. Interestingly, these kinase activities are a result not of mutation (6) but rather of pathway activation within the tumors themselves. In summary, the observation that similar tyrosine kinase activities are present in most if not all anatomically disparate metastatic lesions from the same patient reveals that (*i*) CRPC lesions may be clonal in origin and (*ii*) kinase activation patterns observed in these lesions should be prioritized for further evaluation as new targeted therapeutic strategies.

Results

Phosphotyrosine Peptide Signatures Are Dramatically Different Between Prostate Cancer Cell Line-Derived Xenografts and Treatment-Naïve or Metastatic CRPC Tissues. To identify and discover unique kinase targets in metastatic CRPC, we analyzed 16 metastatic CRPC samples from 13 different patients obtained at rapid autopsy (15) by quantitative label-free phosphotyrosine MS (Fig. 1). These included eight anatomically unique sites as well as two or three distinct sites from three separate patients. Each sample contained greater than 50% tumor content as determined by histological analyses. We also analyzed one benign prostatic hyperplasia (BPH), six treatment-naïve matched benign and cancerous prostates, and metastatic or s.c. xenograft tumors derived from the androgen-insensitive 22Rv1 and androgen-sensitive LNCaP cell lines (Dataset S1) (8). From three separate phosphotyrosine enrichment preparations and MS analyses, we identified 297 unique phosphopeptides corresponding to 185 unique proteins (Dataset S2).

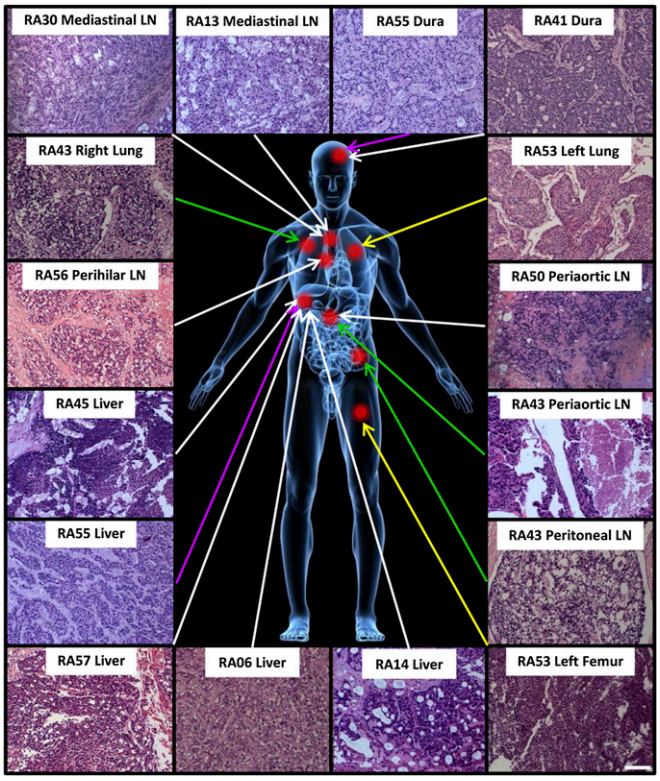

Fig. 1. Anatomical location and histological characterization of metastatic CRPC samples used for phosphoproteomics. Metastatic CRPC tissues were obtained from the Rapid Autopsy Program at the University of Michigan. Sixteen samples from 12 different patients are represented and prepared as previously described for phosphoproteomics (8). Red dots indicate the approximate location of the metastatic lesions analyzed. Same-colored lines represent tissues from the same patient. Patient RA53 left lung and left femur were combined due to limiting material (yellow lines). Only tissues with greater than 350 mg and 50% tumor content were evaluated. (Scale bar, 50 μm.)

To compare different models and stages of prostate cancer, we included cell line-derived xenografts, treatment-naïve primary prostate benign and cancerous tissues, and metastatic CRPC in a single phosphotyrosine enrichment preparation. Unsupervised hierarchical clustering revealed three separate clusters. In particular, the cell line-derived xenografts formed a distinct group compared to the primary tissues, indicating that these xenografts are poor representations of primary patient tissue (Fig. 2*A*). In addition, unsupervised hierarchical clustering also did not distinguish between the patient-matched benign or cancerous prostates, indicating that tyrosine phosphorylation remains relatively unchanged in treatment-naïve benign or cancerous prostates (Fig. 2*A* and Figs. S1 and S2). This suggests that evaluation of phosphotyrosine activity in metastatic CRPC tissues is crucial to testing potential new therapeutic treatments.

Phosphoproteomic Profiling and Kinase/Substrate Enrichment Analyses Identifies Several Druggable Nonmutated Kinase Targets and Pathways in Metastatic CRPC Lesions. Most patients with metastatic CRPC present with metastases at multiple sites, creating a therapeutic dilemma (15). We set out to examine heterogeneity in a cohort of metastatic CRPC patients including those with multiple, anatomically distinct metastatic sites for activated kinase targets. Several metastatic CRPC patients that we evaluated contained similar anatomic sites of involvement including tumors in the liver, lung, dura, and distant lymph nodes. Unsupervised hierarchical clustering of the tyrosine phosphorylation patterns of 10 metastatic lesions, including two patients for which we had two independent metastatic lesions, grouped samples by both patient and metastatic site (Fig. 2*B* and Fig. S3).

Phosphotyrosine peptide identification directly identified several activated kinases and phosphatases [tyrosine kinase 2 (TYK2) Y^{292}, protein tyrosine kinase 2 beta (PTK2B) Y^{579}, MAPK1/3 $Y^{187/204}$, discoidin domain receptor tyrosine kinase 1 (DDR1) Y^{796}, the JAK2/SRC kinase target STAT3 Y^{705}, and protein tyrosine phosphatase, non-receptor type 11 (PTPN11) $Y^{62/63}$]. Kinase–substrate relationship analyses, which predict kinase activity based on phosphopeptide motifs (8), have also identified putative upstream kinases and phosphatases [anaplastic lymphoma kinase (ALK), EGFR, PTK6, SRC, and PTPN2] that were active in individual metastatic CRPC samples (Figs. S1–S3 and Datasets S3–S5). These identifications were notable because of the US Food and Drug Administration–approved late-stage clinical trial of available kinase inhibitors targeting SRC (dasatinib/bosutinib/ponatinib) (16–18), EGFR (erlotinib) (19), ALK (crizotinib) (20), the MAPK1/3 upstream pathway kinases mitogen-activated protein kinase kinase 1/2 (MEK1/2) (trametinib) (21), or the STAT3 upstream kinase JAK2 (ruxolitinib) (22). Western blot analyses from five different patients confirmed the activation states of some of these kinases and also revealed interpatient heterogeneity as each patient evaluated displayed a unique phosphopattern (Fig. 2*C*). As expected, when evaluating prospectively the mutational status of a subset of our samples, we observed little to no activating mutations in these kinases. We did find one patient, RA57 Liver, to have two mutations [one in ephrin type-A receptor 4 (EPHA4) and one in mast/stem cell growth factor receptor (SCFR or KIT)] (6). However, our kinase/substrate enrichment scores did not predict kinase activity of either EPHA4 or KIT, again suggesting

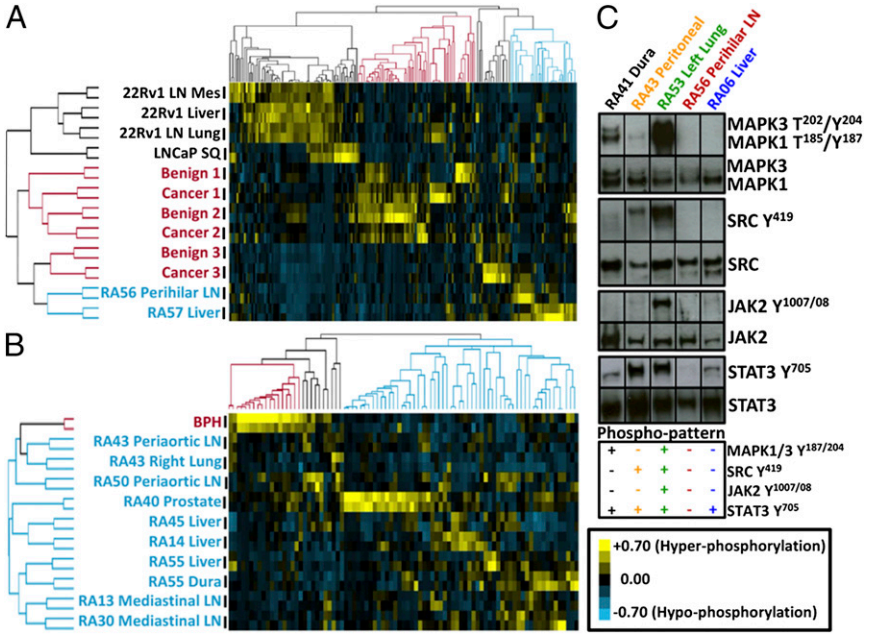

Fig. 2. Phosphoproteomic analyses of cell line-derived xenografts, treatment-naïve prostate cancer, and metastatic CRPC reveal distinct phosphopatterns. (*A*) Unsupervised hierarchical clustering of phosphotyrosine-enriched peptides separates cell line-derived xenograft tumors from primary prostate or metastatic tissue. (*B*) Further evaluation of a separate run of 10 metastatic CRPC lesions reveals patient-specific and metastatic site similarity of phosphotyrosine peptide patterns. (*C*) Western blot validation of four different activated kinases identified from both phosphoproteomics and inferred kinase activities confirms the heterogeneity observed across five different patients, as each patient exhibited a unique phosphopattern. Western blot data were separated to highlight each individual patient but were performed on the same western blot. Yellow, hyperphosphorylation; blue, hypophosphorylation. Intensity bar in Fig. 2*B* is applicable to Fig. 2*A*.

that these mutations did not lead to any detectable levels of activation of these kinases in this tissue sample.

Correlation analysis of the phosphotyrosine signaling patterns revealed a significant level of similarity in the phosphotyrosine profiles from lesions derived from a single patient, despite the fact that these lesions were derived from distinct anatomical sites (Fig. S4). Comparing three liver metastases, we also observed high levels of similarity between two of three lesions (Fig. S4). These MS–based phosphoproteomic data suggest that metastatic CRPC lesions isolated from the same patient may exhibit highly similar tyrosine kinase activation patterns but do not exclude the possibility that anatomical location may also drive similar phosphotyrosine signaling patterns in CRPC. This aspect is further analyzed below.

Large-Scale Analyses of Kinase Activation Patterns Reveals Intrapatient Similarity Across Multiple, Anatomically Distinct Metastases. To determine if signaling patterns were more similar within anatomically distinct metastatic lesions from the same CRPC patient or within sites of metastasis, we examined a larger, independent set of patients that included 28 distinct metastatic lesions from seven different CRPC patients (Fig. S5). Western blot analysis of phosphoproteins identified by MS and kinase/substrate enrichment analysis or the activated states of receptor tyrosine kinase (RTK) targets [EGFR Y^{1173}, ERBB2 Y^{1221}, and hepatocyte growth factor receptor (HGFR or MET) Y^{1234}] for which there are clinical inhibitors available confirmed our initial observation of intrapatient similarities (Fig. 3 and Fig. S6 *A*–*C*). Comparison of different patients revealed dramatically different kinase activation patterns. This ranged from SRC Y^{419}, STAT3 Y^{705}, MAPK1/3 $T^{185/202}/Y^{187/204}$, and AKT S^{473}, activated upon phosphatase and tensin homolog (*PTEN*) loss in the majority of prostate cancers, for patient RA43 to only STAT3 Y^{705} for patient RA55 (Fig. 3). These unique phosphopatterns suggest that shared kinase activities exist in metastatic CRPC lesions isolated from the same patient.

To determine if this pattern of intrapatient similarity across metastases remains consistent with a larger set of other RTK and intracellular kinases, we evaluated five previously analyzed sets of patient metastases using RTK and phosphokinase arrays from R&D Systems. Analysis of three or four anatomically distinct metastatic lesions from each patient revealed signaling patterns that were qualitatively similar within a patient's set of metastatic lesions (Fig. 4*A*). Patient-specific patterns included (*i*) tyrosine phosphorylation of ALK, RYK, and the activation site of AKT T^{308} in patient RA37; (*ii*) hemopoietic cell kinase (HCK) pY^{411} from patient RA56; and (*iii*) cellular RET (c-RET) phosphorylation in RA33 (Fig. 4*A*). Quantitation of these arrays revealed intrapatient similarities for nine phospho- and total proteins (Fig. 4*B*). Principal component analysis (PCA) of the kinases and proteins with detectable phosphorylation or expression ($n = 11$) demonstrated highly similar intrapatient grouping (Fig. 4*C* and Fig. S7). Surprisingly, the signaling patterns found in these metastatic lesions appear to be substantially cell autonomous as lesions from similar anatomical sites did not group together (Fig. 4*D*). Statistical analysis of pairwise correlation coefficients confirmed that metastatic CRPC lesions isolated from the same patient have strongly similar signaling patterns, more so than lesions from similar anatomical sites in different patients (Fig. S8).

Phosphorylation of Neuronal RTK RET in Metastatic CRPC Lesions with a Small Cell Neuroendocrine Carcinoma Phenotype. Further evaluation of the phospho-RTK arrays revealed tyrosine phosphorylation of RET in patient RA33 (Fig. 3*A*). RET is expressed in neuronal cell types, suggesting this patient may have suffered from a rare small cell neuroendocrine carcinoma (SCNC) phenotype (23). Indeed histological analyses of patient RA33 confirmed SCNC as evidenced by a diffuse, solid growth pattern with darkly stained nucleus, a homogeneous chromatin pattern, high nuclear/cytoplasmic (N/C) ratio, lack of nucleoli, and frequent mitotic figures (Fig. S9 *A* and *B*, arrows). These are in sharp contrast

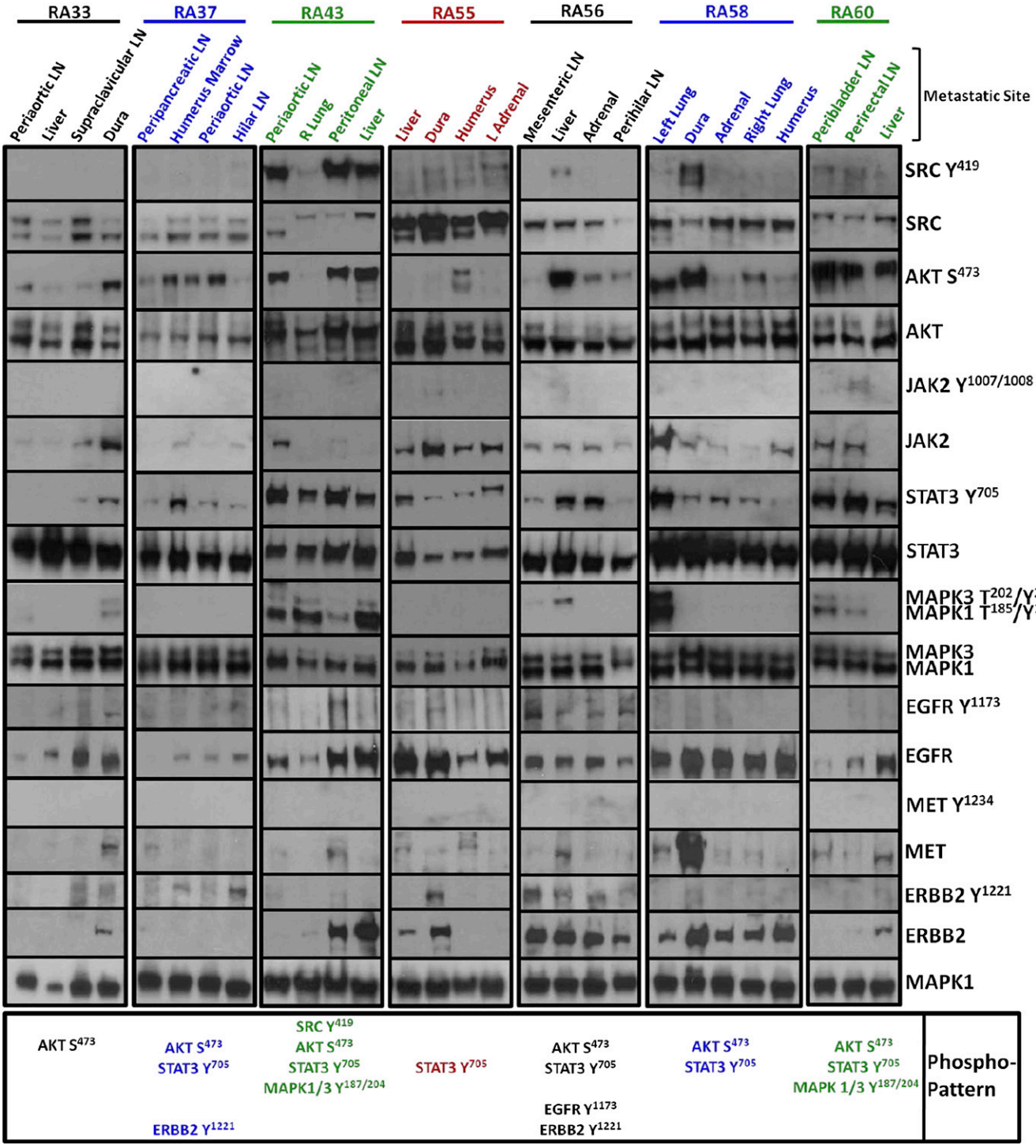

Fig. 3. Related phosphokinase and substrate expression patterns are observed within distinct anatomical metastatic lesions of the same patient. Western blot analyses from seven different sets of patients with three or four distinct metastatic lesions were evaluated for kinase activation patterns that were identified in the phosphoproteomic datasets and kinase–substrate relationships or RTKs that have been previously targeted clinically. Each patient expressed similar activated kinase patterns independent of the anatomical location of the metastatic lesions. The unique phosphopatterns are also depicted schematically below the Western blot data.

to the conventional prostatic adenocarcinoma that shows glandular formation (Fig. S9*C*, dashed circle), nuclear morphology consisting of open and vesicular chromatin patterns, and prominent nuclei (Fig. S9*C*, arrow). These data suggest that the molecular phenotyping of SCNC, as indicated by phospho-RET activity, may drive novel therapeutic strategies for this rarer subtype of prostate cancer.

Stratification of Metastatic CRPC Patients' Kinase Activation Patterns Suggests That Simultaneous Targeting of SRC and MEK Kinases May Be of Potential Therapeutic Value. To predict potential kinase inhibitor combination therapies for metastatic CRPC patients, we evaluated all 16 individual metastatic CRPC lesions that had been analyzed by phosphoproteomics. We pooled kinases that were

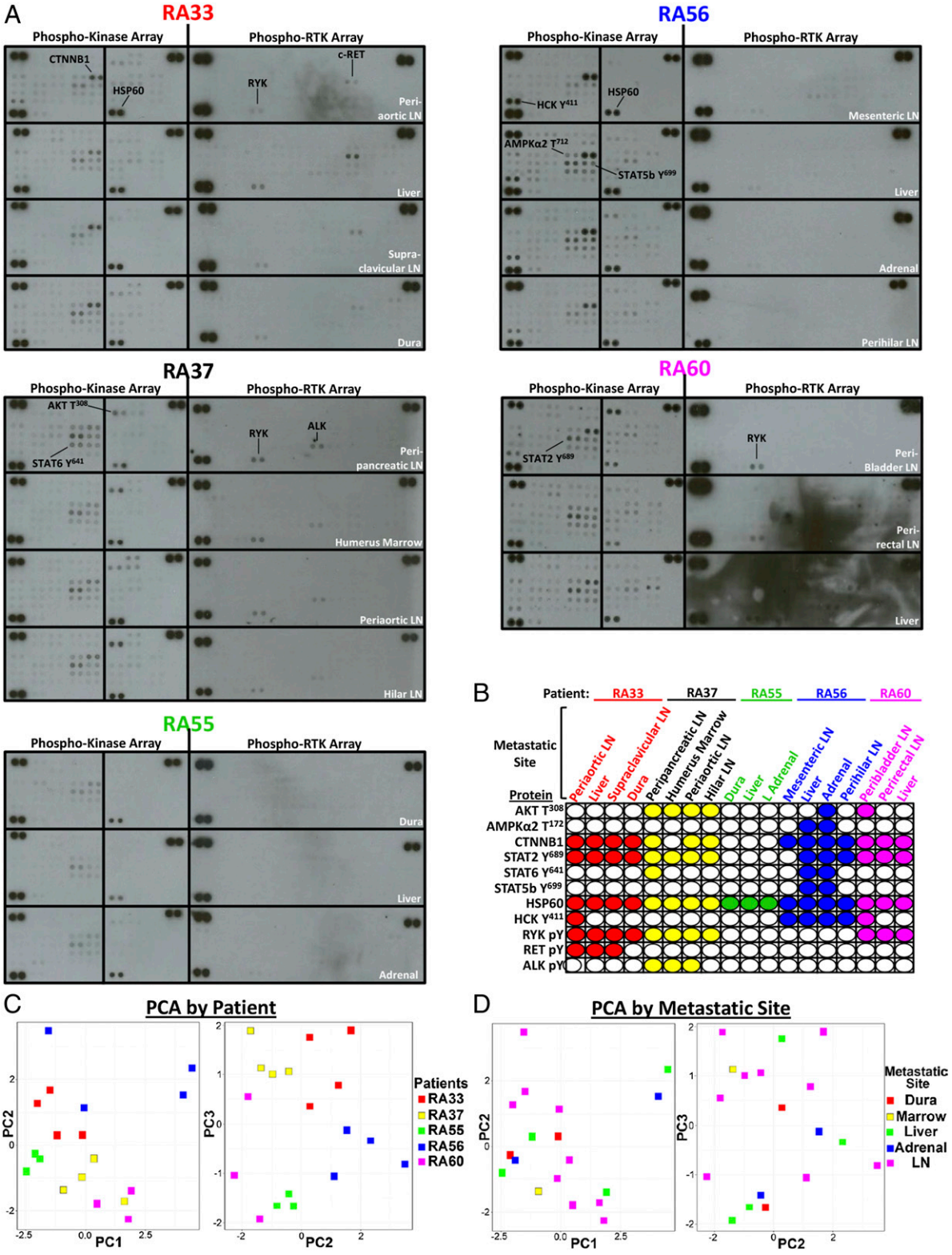

Fig. 4. Large-scale analyses of kinase activation patterns confirm intrapatient similarity across multiple, anatomically distinct metastases. (*A*) Phosphokinase and phospho-RTK arrays were used to analyze metastatic lesions from five different patients from anatomically distinct metastatic lesions. (*B*) Unique phosphopatterns were observed for each patient, and similar patterns were observed within the same patient, as shown with like-colored circles. Each observable phospho- or total protein spot from the phosphokinase and RTK arrays were used for PCA. LN, lymph node. (*C*) PCA analysis of all five patients confirms intrapatient kinase expression similarity and interpatient dissimilarity. (*D*) Grouping metastatic lesions by similar anatomical site shows no significant grouping of samples. Each phosphokinase and phospho-RTK array are spotted in duplicate, and positive control spots are located in the top left, right, and bottom left of each array. The first three principal components represent 77% of the total variance. Adrenal, adrenal gland lesions; LN, distant lymph node lesions; marrow, bone marrow lesion.

Table 1. Kinase and inhibitor stratification of metastatic CRPC patients

Patient number and metastatic location	Identified kinases via MS and western blot plus inferred kinases via kinase–substrate relationships*	Potential clinical inhibitors				
		Dasatinib†	Erlotinib‡	Crizotinib§	Ruxolitinib¶	Trametinib‖
RA06 Liver	EPHA3-7, SRC, PDGFR	X				
RA13 Mediastinal LN	ALK, FLT3/CSF1R/KIT, INSR, MAPK1, MAP3K2, PTK6, SRC	X		X		X
RA14 Liver	EGFR, MAPK1, MAP3K2, PTK6	X	X			X
RA30 Mediastinal LN	ALK, FLT3/CSF1R/KIT, MAP3K2, PTK6, SRC	X		X		X
RA40 Prostate	EGFR, MAPK1/3, MAP2K2, MAP3K2, PTK6, SRC	X	X			X
RA41 Dura	FLT3/CSF1R/KIT, MAPK1/3, SRC	X				X
RA43 Peritoneal and Right Lung	ALK, EGFR, EPHA3-7, MAPK1/3, PTK6, SRC	X	X	X		X
RA43 Periaortic LN	MAPK1/3, SRC	X				X
RA43 Right Lung	EGFR, FLT3/CSF1R/KIT, MAPK1/3, MAP2K2	X	X			X
RA45 Liver	ALK, MAP3K2			X		X
RA50 Periaortic LN	MAPK1/3, MAP3K2					X
RA53 Left Femur and Left Lung	ALK, EPHA3-7, JAK2, MAPK1/3, PDGFR, PTK6, SRC	X		X	X	X
RA55 Liver	ALK, EGFR, EPHA3, MAPK1/3, MAP2K2, MAP3K2, PTK6	X	X	X		X
RA55 Dura	EGFR, PTK6	X	X			
RA56 Perihilar LN	EGFR, HCK, TYK2	X	X		X	
RA57 Liver	EPHA7, MAP3K2, TYK2	X			X	X

*Kinases corresponding to identified phosphopeptides observed as >twofold over benign tissues, via western blotting, or kinase–substrate relationships ($P < 0.1$) as shown in Dataset S4.
†SRC family kinase, KIT, PDGFR, and EPHA receptor inhibitor.
‡EGFR inhibitor.
§ALK inhibitor.
¶JAK2 inhibitor.
‖MEK inhibitor.

identified from MS, western blot, and predicted kinase–substrate relationships to reveal a wide range of predicted kinase activities across the patient samples (Table 1). Mapping clinically available inhibitors to these kinases revealed 11 different TKI combinations with overlap between four sets of inhibitor combinations (Table 1). Notably, the SRC inhibitor dasatinib and the MEK inhibitor trametinib were predicted therapeutic strategies in 14 of 16 (87.5%) or 13 of 16 (81.2%) patients, respectively. If we consider combination therapy, 11 of 16 (68.8%) patients would be predicted to benefit from both SRC and MEK inhibitors, whereas 5 of 16 (31.2%) patients would not. There are no current clinical trials in prostate cancer evaluating the efficacy of SRC and MEK combination therapy in metastatic CRPC, but if initiated, stratification of patients based on activation of these two kinases would be necessary. Overall, the kinases identified in metastatic CRPC patients using phosphoproteomic analyses (*i*) may guide the molecular stratification of patients to direct the proper course of treatment with kinase inhibitor combinations, (*ii*) confirm the complexity observed across patients, and (*iii*) suggest that individualized therapy needs to be considered before clinical treatment decisions.

Discussion

From our study, we were able to measure protein phosphorylation in 41 metastatic CRPC samples from 17 patients including 16 samples by quantitative phosphotyrosine MS. Our phosphokinase profiling and evaluation of active kinases suggests that kinase activity patterns are patient-specific and are maintained across multiple metastatic lesions within the same patient. These data support previous studies suggesting that metastatic disease arises from a single precursor cancer cell or focal mass located at the primary tumor site (6, 12–14). Our findings add actionable information to this perspective. Kinase inhibitor treatment regimens guided by the biopsy of a single accessible metastatic lesion may be sufficient to predict the responses of multiple sites, leading to a more efficacious use of single agents or multidrug combinations, although this concept is still untested.

The development of new targeted therapies for metastatic CRPC presents a number of clinical questions. Major challenges include effective stratification of patients who will benefit from selected treatments and recognition of context-specific molecular targets. One approach to address these issues is the serial sampling and molecular characterization of malignant tissue from patients during the course of their disease. The increasing availability of high-throughput tools has enabled the genomic and transcriptomic profiling of large numbers of clinical carcinoma samples of different subtypes (4, 24, 25). Phosphoproteomic technology, particularly mass spectroscopy–based proteomics, is also rapidly advancing and has recently been applied to the elucidation of tyrosine-kinase–driven pathways in cell lines (26–29) or the discovery of activated kinases that may be useful for therapy in human cancers (30, 31).

Our analysis of phosphotyrosine signaling patterns in primary tumors and xenografts indicates that the prostate cell line-derived xenografts evaluated have different phosphorylation patterns compared with primary tissues. Supporting this notion, gene expression studies in small-cell lung cancer (SCLC) also identified primary tumor-specific signatures that were lost upon transitioning to cell culture (32), and proteomic analyses in colorectal cancer suggest that xenograft tumors are dramatically different from their cell line counterparts (33). This suggests that the stratification and prioritization of therapeutic targets for CRPC will require analysis of primary tissue, rather than cell lines or cell line-derived xenografts.

Interestingly, very few patient sets were positive for the activated states of EGFR, ERBB2, or MET, although they were detected in prostate cancer cell lines. Drugs targeting EGFR and ERBB2 did not produce significant results in CRPC patients (34,

35), however the MET inhibitor cabozantinib has shown promise in the clinic (36). This is in contrast to our observation that MET activity is not detected in our analyzed metastatic CRPC tissues. One explanation is that our sampling of metastatic CRPC tissues is too small or that MET activity was lost before tissue collection and we were not able to detect it. Two other possibilities are that cabozantinib activity in metastatic CRPC is not targeted toward epithelial MET but rather to MET expressed in osteoblasts or other mesenchymal cells in the bone microenvironment (36) and that cabozantinib is inhibiting another tyrosine kinase such as VEGFR2 or RET (37). Although we did not evaluate VEGFR2 activity, we did observe RET activity in SCNC, suggesting this kinase may be potentially targeted by cabozantinib in metastatic CRPC patients.

Rapid autopsy programs have paved the way for studies in genomic mutations, copy number alterations, and splicing variants from metastatic tissues that are otherwise difficult to obtain (4, 6, 15, 38, 39). Although we evaluated many soft tissue metastatic lesions, we were only able to evaluate five bone metastases. Although bone metastases are evident in over 90% of metastatic CRPC patients (15), metastatic bone tumors are hard to study because tumor material is lodged into hard, calcified bone, preventing the procurement of quality material for analysis. This is also especially difficult considering the large amount of tissue (>350 mg) required for phosphoproteomic preparations. A potential outcome could be that kinase patterns are principally determined by site of metastasis due to signals initiated by the surrounding local microenvironment creating a premetastatic niche (40). Tissue-specific kinase activation patterns were not observed in our study, but further evaluation of bone metastases in patients also harboring soft tissue metastases will be necessary to extend these findings.

Materials and Methods

Tissue Culture of Prostate Cancer Cell Lines and Derivation of Xenograft Tumors. 22Rv1 cells were grown in RPMI medium supplemented with L-glutamine, FBS, and nonessential amino acids (NEAAs). LNCaP, DU145, and C4-2 cells were grown in DMEM supplemented with L-glutamine, FBS, and NEAA. Thirty 15-cm plates were collected from each cell line and treated with 2 mM Vanadate for 30 min. Cells were subsequently lysed in 9 M Urea lysis buffer and used for phosphoproteomic analysis.

To generate metastatic tumors, 1×10^5 22Rv1 cells were injected intracardially as previously described, and dissemination was monitored using bioluminescence imaging (41). After 8 wks, tumors were extracted from the metastatic locations including the liver and lymph nodes in the mesenteric and lung regions. Also, to evaluate primary tumor growth, 1×10^6 LNCaP cells were injected s.c. and excised once they reached Division of Laboratory Animal Medicine (DLAM) limits.

Acquisition of Clinically Matched Benign and Cancerous Primary Prostate Tissues and Metastatic CRPC Samples. Patient samples were obtained from the University of California–Los Angeles (UCLA) Translational Pathology Core Laboratory, which is authorized by the UCLA Institutional Review Board to distribute anonymized tissues to researchers as described previously (42–44). Cancer and benign areas were clearly marked on the frozen section slides, and prostate tissue containing the cancer region was separated from the benign area before collecting for phosphoproteomic analyses.

The Rapid Autopsy program at the University of Michigan has been previously described (11, 39). Frozen tissues from the Rapid Autopsy program were sent overnight on dry ice for phosphotyrosine peptide analysis. Sections were stained with hematoxylin and eosin for representative histology.

Quantitative Analysis of Phosphotyrosine Peptides by MS. Tissue lysis was performed as previously described (8). Briefly, greater than 350 mg of frozen tumor mass was homogenized and sonicated in urea lysis buffer (20 mM Hepes pH 8.0, 9 M urea, 2.5 mM sodium pyrophosphate, 1.0 mM beta-glycerophosphate, 1% *N*-octyl glycoside, 2 mM sodium orthovanadate). Total protein was measured using the bicinchoninic acid (BCA) Protein Assay Kit (Thermo Scientific/Pierce), and 25 mg of total protein was used for phosphoproteomic analysis. The remaining protein lysate was frozen for subsequent western blot analyses.

Phosphotyrosine peptide enrichment and liquid chromatography tandem MS (LC-MS/MS) analysis was performed as previously described (8, 26, 45). Phosphopeptides were identified using the Proteome Discoverer software (version 1.4.0.88, Thermo Fisher Scientific). MS/MS fragmentation spectra were searched using SEQUEST against the Uniprot human reference proteome database with canonical and isoform sequences (downloaded January 2012 from uniprot.org). Search parameters included carbamidomethyl cysteine (*C) as a static modification. Dynamic modifications included phosphorylated tyrosine, serine, or threonine (pY, pS, and pT, respectively) and oxidized methionine (*M). The Percolator node of Protein Discoverer was used to calculate false discovery rate (FDR) thresholds, and the FDR for the datasets was adjusted to 1% (version 1.17, Thermo Scientific). The Percolator algorithm uses a target-decoy database search strategy and discriminates true and false identifications with a support vector machine (46). The PhosphoRS 2.0 node was used to more accurately localize the phosphate on the peptide (47). Only phosphopeptides with at least one phosphotyrosine assignment with a reported probability above 20% were considered. MS2 spectra for all reported phosphopeptides are deposited to the ProteomeXchange Consortium with the dataset identifier PXD000238 (48).

Data Analysis. Data analysis was performed as previously described (8). For clustering, we removed any peptides that had an ANOVA score greater than 0.2. Hierarchical clustering of phosphotyrosine data was performed using the Cluster program with the Pearson correlation and pairwise complete linkage analysis (49) and visualized using Java TreeView (50). Quantitative data for each phosphopeptide can be found in Dataset S5, *Batch 1–3*. To evaluate the significance of intrapatient and anatomical site similarity, the Pearson correlation coefficient was calculated for each pair of phosphotyrosine samples, and the resulting correlation matrix was clustered using the pHeatmap package in R. Statistical significance was assessed against the null hypothesis that the correlation was not different from zero.

Prediction of Kinase–Substrate Relationships and Enrichment Analysis of Kinase Activity. Predictions, enrichment, and permutation analyses have been previously described (8). Phosphotyrosine peptides were ranked by the signal-to-noise ratio observed for a given perturbation (e.g., metastatic CRPC compared with benign prostate or BPH). The enrichment scores for all putative upstream kinases are shown in Dataset S4, *Batch 1–3*.

Western Blot. For western blots, equal protein amounts of metastatic CRPC tissue urea lysates (20 or 30 μg) were used from tissues prepared as described previously (8). Antibodies were diluted as follows: AKT (1:1,000, Santa Cruz), pAKT S^{473} (1:2,000, Cell Signaling), EGFR (1:1,000, Cell Signaling), pEGFR Y^{1173} (1:1,000, Cell Signaling), STAT3 (1:1,000, Cell Signaling), pSTAT3 Y^{705} (1:2,000, Cell Signaling), JAK2 (1:1,000, Cell Signaling), pJAK2 $Y^{1007/1008}$ (1:500, Cell Signaling), MAPK1/3 (1:1,000, Cell Signaling), MAPK1/3 $T^{185/202}$/$Y^{187/204}$ (1:2,000, Cell Signaling), SRC (1:1,000, Millipore), pSRC Y^{419} (1:1,000, Cell Signaling), ERBB2 (1:1,000, Cell Signaling), pERBB2 $Y^{1221/1222}$ (1:1,000, Cell Signaling), MET (1:1,000, Cell Signaling), and pMET Y^{1234} (1:1,000, Cell Signaling). ECL substrate (Millipore) was used for detection and development on GE/Amersham film.

Phospho-RTK and Phosphokinase Arrays. Human Phospho-RTK (R&D Sytems) and phosphokinase (R&D Systems) arrays were used according to the manufacturer's instructions. Briefly, 300 μg of 9 M urea lysate for each metastatic sample was diluted in the kit-specific dilution buffer to a final concentration of 0.85 M urea and incubated with blocked membranes overnight. The membranes were washed and exposed to chemiluminescent reagent and developed on GE/Amersham film. Quantitation of each array was performed using Image J. To evaluate the significance of intrapatient and anatomical site similarity, the Pearson correlation coefficient was calculated for each pair of samples using only the kinases and proteins with detectable phosphorylation or expression (n = 11), and the correlation coefficients were clustered using the pHeatmap package in R. Statistical similarity of intrapatient lesions was assessed against the null hypothesis that the correlation was not different from zero. P values from multiple comparisons were combined using Fisher's Method where appropriate.

PCA. Each antibody-related spot on the Phospho-RTK and phosphokinase arrays was quantified using Image J. After background subtraction, the duplicate spots for each antibody were averaged, and antibodies with negligible signal were removed. The data were unit normalized, and principal components were calculated in R.

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U.S. Provisional Patent Application for:
IDENTIFICATION OF PHOSPHOPEPTIDES IN PROSTATE CANCER FOR THERAPEUTIC TREATMENT

Inventor(s): Owen Witte, Justin M. Drake, Nicholas A. Graham, and Thomas G. Graeber

Dear UCLA/OIP:

Enclosed for your files are copies of the Provisional Application Cover Sheet and Provisional Patent Application, which were filed with the U.S. Patent and Trademark Office on June 19, 2013.

Also enclosed for your records is a copy of the Electronic Acknowledgement Receipt which confirms the application was filed on June 19, 2013 and received serial no. 61/836,984.

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Very truly yours,

William J. Wood

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Provisional Patent Application

for:

IDENTIFICATION OF PHOSPHOPEPTIDES IN PROSTATE CANCER FOR THERAPEUTIC TREATMENT

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IDENTIFICATION OF PHOSPHOPEPTIDES IN PROSTATE CANCER FOR THERAPEUTIC TREATMENT

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This invention was made with Government support under Grant No. W81XWH-11-1-0504, awarded by the U.S. Army Medical Research and Materiel Command. The Government has certain rights in this invention.

TECHNICAL FIELD

Methods and compositions useful for monitoring polypeptide phosphorylation patterns associated with cancers such as prostate cancer.

BACKGROUND OF THE INVENTION

Worldwide, prostate cancer is the fourth most prevalent cancer in men. In North America and Northern Europe, it is by far the most common male cancer and is the second leading cause of cancer death in men. In the United States alone, well over 40,000 men die annually of this disease - second only to lung cancer. Despite the magnitude of these figures, there is still no effective treatment for metastatic prostate cancer. Surgical prostatectomy, radiation therapy, hormone ablation therapy, and chemotherapy continue to be the main treatment modalities. Unfortunately, these treatments are ineffective for many and are often associated with undesirable consequences.

Prostate cancer lacks effective treatments. On the diagnostic front, the lack of a prostate tumor marker that can accurately detect early-stage, localized tumors remains a significant limitation in the management of this disease. Although the serum PSA assay has been a very useful tool, its specificity and general utility is widely regarded as lacking in several important respects. While previously identified markers such as PSA have facilitated efforts to diagnose and treat prostate cancer, there is need for the identification of additional markers and therapeutic targets for prostate and related cancers in order to further improve diagnosis and/or therapy.

SUMMARY OF THE INVENTION

As disclosed herein, we identify phosphorylation sites on various cellular peptides that predict aspects of treatment efficacy for prostate cancer in lieu of activating mutations or DNA amplifications. These phospho-peptides further identify numerous kinase activities which then provide complementary information about activation states of proteins that DNA sequencing or other conventional genetic approaches miss.

As discussed in detail below, we have identified phosphopeptides from metastatic castration resistant prostate cancer that are targets for therapy. The phosphopeptide identifications and subsequent kinase/substrate motif analyses from metastatic castration resistant prostate cancer samples provide novel targets for therapy. We identified 297 unique phosphopeptides corresponding to 185 unique proteins, many of which may be a novel target for therapy in prostate cancer. Publications describing methods and materials that can be used to adapt this technology for other cancers include, for example, Rikova et al. Cell, 2007 Dec 14;131(6):1190-203, PubMed ID:18083107. Procedures of phosphotyrosine enrichment and certain phosphopeptides are disclosed for example in US Patent Application No. 20030044848 and US Patent No. US797746). The connection of tyrosine phosphorylated-RET in Small Cell Carcinoma of the prostate is novel. In addition, we have identified other kinase activities that if inhibited in combination could be novel, effective therapies for incurable late stage prostate cancers.

This discoveries disclosed herein can be used in the diagnosis, prognosis and therapy of patients with metastatic castration resistant prostate cancer. For example, if a single patient expressed 2 different kinases then the administration of 2 different kinase inhibitors would be recommended. This idea can be carried forward for other patients that express different combinations of activated kinases.

Other objects, features and advantages of the present invention will become apparent to those skilled in the art from the following detailed description. It is to be understood, however, that the detailed description and specific examples, while indicating some embodiments of the present invention are given by way of illustration and not limitation. Many changes and modifications within the scope of the present invention may be made without departing from the spirit thereof, and the invention includes all such modifications.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Anatomical location and histological characterization of metastatic castration resistant prostate cancer (CRPC) samples used for phospho-proteomics. Greater than 350 mg of metastatic CRPC tissues were obtained from the Rapid Autopsy Program at The University of Michigan. 16 samples from 12 different patients are represented and prepared as previously described for phospho-proteomics (*8*). Red dots indicate the approximate location of the metastatic lesions analyzed. Same-colored lines represent tissues from the same patient. Patient RA53 left lung and left femur were combined due to limiting material (yellow lines). Only tissues with greater than 50% tumor content were evaluated. Scale bar=50 μm.

Figure 2. Phosphoproteomic analyses of metastatic CRPC exhibits inter-patient heterogeneity and intra-patient similarity. a. Unsupervised heirarchal clustering of phospho-tyrosine enriched peptides separates cell line-derived xenograft tumors from primary prostate or metastatic tissue. b. Further evaluation of a separate run of 10 metastatic CRPC lesions reveals patient-specific similarity of phospho-tyrosine peptide patterns (red rectangles) while distinct phospho-tyrosine peptide patterns are unique for each patient. c. Western blot validation of 4 different activated kinases identified from both phospho-proteomics and inferred kinase activities confirms the heterogeneity observed across 5 different patients as each patient exhibited a unique phospho-pattern. Western blot data was separated to highlight each individual patient but were performed on the same western blot. Yellow=hyperphosphorylation, Blue=hypophosphorylation. Intensity bar in Fig. 2b is applicable to Fig. 2a.

Figure 3. Related phospho-kinase and substrate expression patterns are observed within distinct anatomical metastatic lesions of the same patient. Western blot analyses from 7 different sets of patients with 3 or 4 distinct metastatic lesions were evaluated for kinase activation patterns that were identified in the phospho-proteomic data sets and kinase/substrate relationships or receptor tyrosine kinases that have been previously targeted clinically. Each patient expressed similar activated kinase patterns independent of the anatomical location of the metastatic lesions. The unique phospho-patterns are also depicted schematically below the western blot data.

Figure 4. Large scale analyses of kinase activation patterns confirms intrapatient similarity across multiple, anatomically distinct metastases. a. Phospho-kinase and phospho-RTK arrays were analyzed from 5 different patients that display anatomically distinct metastatic sites. b. Unique phospho-patterns were observed for each patient and similar patterns were observed within the same patient as shown with like-colored circles. Each observable phospho- or total protein spot from the phospho-kinase and RTK arrays were used for principal component analysis (PCA). c. PCA analysis of all 5 patients confirms intra-patient kinase expression similarity and individual differences. Each phospho-kinase and phospho-RTK array are spotted in duplicate and positive control spots are located in the top left, right, and bottom left of each array. Each phospho-array was quantified using Image J.

Supplementary Figure 1. Phosphoproteomic analyses exhibits distinct clusters of phosphorylation between the cell line-derived xenografts and primary prostate tissues. (A) Unsupervised heirarchal clustering does not group cell line-derived metastatic xenograft tumors with either organ confined or metastatic CRPC. Also, treatment naïve patient matched benign and cancerous prostates display indistinguishable phospho-peptide signatures. The phospho-profiling heatmap from Figure 2a with the protein and residue identities of the phosphorylation events are listed. (B) Unsupervised heirarchal clustering does not group by organ site of metastases but rather by intra-patient metastatic lesions. Benign prostatic hyperplasia (BPH) was used as the treatment naïve tissue for comparison. The phospho-profiling heatmap from Figure 2b with the protein and residue identities of the phosphorylation events are listed. (C) Unsupervised heirarchal clustering does not group organ confined prostate benign or cancerous prostates with metastatic CRPC. Also, treatment naïve patient matched benign and cancerous prostates display indistinguishable phospho-peptide signatures. The phospho-profiling heatmap from Batch 2 with the protein and residue identities of the phosphorylation events are listed. For all heatmaps, the labels are as follows: UniProt ID, phosphosite residue number, phospho-peptide (charge state of mass spectrometry ion). If the phospho-peptide has multiple identities, a slash separates each protein and phosphorylation residue number. The vertical line separates the proteins from the phospho-peptide. Yellow=hyperphosphorylation, Blue=hypophosphorylation.

Supplementary Figure 2. Location and histological characterization of 7 patients

with anatomically distinct metastatic castration resistant prostate cancer (CRPC) lesions. 7 separate patients' metastatic lesions are depicted with representative histology. These samples were used for western blot and phospho-RTK and phospho-kinase arrays. Red dots indicate the approximate location of the metastatic lesions analyzed. Tissues with greater than 50% tumor content were evaluated. Scale bar=50 μm.

Supplementary Figure 3. Evaluation of receptor tyrosine kinase (RTK) EGFR, ERBB2, and MET and phospho-kinase and phospho-RTK arrays using positive control prostate cancer cell lines. Western blot analyses from DU145 or 22Rv1 cells treated with the phosphatase inhibitor, vanadate, were evaluated for the activated states of the receptor tyrosine kinases (RTKs) EGFR, ERBB2, and MET (A), phospho-kinase (B), or phospho-RTK arrays (C). DU145 or 22Rv1 (indicated by an asterisk next to the blot) cells were used as positive controls.

Supplementary Figure 4. Principal Component Analysis of Phospho-Kinase arrays. Data from CRPC metastatic samples analyzed by phospho-kinase arrays was subjected to principal component analysis. After removal of antibodies with negligible signal, 11 antibodies remained: Akt T^{308}, AMPKa T^{172}, β-catenin, HCK Y^{411}, STAT2 Y^{689}, STAT5b Y^{699}, STAT6 Y^{641}, RYK phospho-tyrosine, RET phospho-tyrosine, and ALK phospho-tyrosine. (A) Schematic of the loadings vectors for the first three principal components. (B) The percentages listed for each principal component (PC) indicated the amount of variance explained by that PC. (C) Plots of the PCA for all 5 patients analyzed demonstrate intra-patient kinase expression similarity and individual differences.

Supplementary Figure 5. Tyrosine phosphorylation of receptor tyrosine kinase (RTK) RET in small cell neuroendocrine carcinoma (SCNC). (A) Analysis of patient RA33 using RTK arrays revealed the tyrosine phosphorylation of neuronal tyrosine kinase RET. (B) Metastatic tumor cells in this patient demonstrate typical nuclear morphology of SCNC including a darkly stained nucleus with a homogeneous chromatin pattern, high nuclear/cytoplasmice (N/C) ratio, lack of nucleoli, and frequent mitotic figures (B, arrows). These characteristics are in sharp contrast to the nuclear morphology of adenocarcinoma tumor cells (C) that have open and vesicular chromatin patterns and prominent nuclei (C, arrow) and glandular formation (C, dashed circle). Scale bar=25 μm.

DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. Many of the techniques and procedures described or referenced herein are well understood and commonly employed using conventional methodology by those skilled in the art. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. Publications cited herein are cited for their disclosure prior to the filing date of the present application. Nothing here is to be construed as an admission that the inventors are not entitled to antedate the publications by virtue of an earlier priority date or prior date of invention. Further the actual publication dates may be different from those shown and require independent verification. In the description of the preferred embodiment, reference is made to the accompanying drawings which form a part hereof, and in which is shown by way of illustration a specific embodiment in which the invention may be practiced. It is to be understood that other embodiments may be utilized and structural changes may be made without departing from the scope of the present invention.

PHOSPHOPROTEOMIC ANALYSIS REVEALS PATIENT-SPECIFIC KINASE ACTIVATION PATTERNS

ABSTRACT

In prostate cancer multiple metastases from the same patient share similar copy number, mutational status, ETS rearrangements, and methylation patterns supporting their clonal origins. Whether actionable targets such as tyrosine kinases are also similarly expressed and activated in anatomically distinct metastatic lesions of the same patient is not known. We evaluated active kinases using phospho-tyrosine peptide enrichment and quantitative mass spectrometry to identify druggable targets in metastatic castration resistant prostate cancer (CRPC) obtained at rapid autopsy. We identified distinct phospho-peptide patterns in metastatic tissues compared to naive primary prostate tissue and prostate cancer cell line-derived xenografts. Evaluation of metastatic CRPC samples for tyrosine

phosphorylation and upstream kinase targets revealed SRC, EGFR, RET, ALK, and MAPK1/3 and other activities while exhibiting intrapatient similarity and interpatient heterogeneity. This suggests that individualized therapy targeting non-mutated kinases with clinical kinase inhibitors may be an effective strategy in the treatment of metastatic CRPC.

INTRODUCTION

Mutational and copy number analyses from epithelial tumors have identified several activating tyrosine kinase mutations and amplifications, such as EGFR mutations in lung adenocarcinoma and ERBB2 amplification in breast cancer (*1*). The dependence on these tyrosine kinases for tumor growth and survival has led to successful clinical treatment with tyrosine kinase inhibitors (TKIs) (*2, 3*). However, recent genomic analyses of prostate adenocarcinoma revealed that activating tyrosine kinase mutations or amplifications are very rare (*1, 4-6*).

Despite the scarcity of tyrosine kinase amplifications or activating mutations in prostate cancer, tyrosine kinase expression and activity has been shown to play an important role in disease progression. For example, co-expression of wildtype SRC tyrosine kinase and androgen receptor (AR) can synergistically drive the formation of mouse prostate adenocarcinoma (*7*). Evaluation of non-tyrosine kinase initiated mouse models of prostate cancer further identified activation of the non-receptor tyrosine kinases SRC, ABL, and JAK2 (*8*). We also observed increased tyrosine phosphorylation in nearly 50% of castration resistant prostate cancer (CRPC) tissues examined when compared to hormone naïve prostate cancer (*8*). These studies suggest that we should better evaluate metastatic CRPC samples for tyrosine kinase activity which may lead to the identification of new drug targets.

Studies in melanoma and breast cancer have revealed that despite heterogeneity in primary, localized disease, metastases seem to arise from a single precursor cell (*9, 10*). The multifocal nature of organ-confined prostate cancer poses a question as to the clonality of metastatic disease (*11*). Investigation into clonality in metastatic CRPC have found that tumors isolated from anatomically different lesions in the same patient bear similar copy number, mutational status, ETS-rearrangements, and methylation patterns from multiple metastatic lesions supporting their clonal origins (*6, 12-14*). In addition, these studies found a remarkable amount of interpatient heterogeneity, suggesting that individualized medicine

approaches may be necessary to efficiently target the metastatic lesions. Previous observations of intrapatient similarity holds promise with regard to treatment strategies for metastatic CRPC patients by means of systematically attacking the cancer cell clone contributing to disease.

This has led us to investigate whether actionable targets such as tyrosine kinases also maintain similar activation patterns across anatomically distinct metastases from the same patient. With access to valuable metastatic CRPC tissue from the University of Michigan's Rapid Autopsy Program (*15*), we evaluated global tyrosine phosphorylation patterns in lethal metastatic CRPC patients. Phosphotyrosine peptide enrichment and quantitative mass spectrometry identified diverse phosphorylation events in the metastatic tissues when compared to naive primary prostate tissue and prostate cancer cell line-derived xenografts. Validation of activated kinases that were identified via either mass spectrometry or kinase/substrate relationships revealed intrapatient similarity and interpatient heterogeneity across a large panel of targets. Interestingly, these kinase activities are not a result of mutation (*6*) but rather pathway activation within the tumors themselves. In summary, the observation that similar tyrosine kinase activities are present in most if not all anatomically disparate metastatic lesions from the same patient suggests that CRPC patients may benefit from individualized, targeted therapies.

RESULTS

Phospho-tyrosine peptide signatures are dramatically different between prostate cancer cell line-derived xenografts and treatment naïve or metastatic castration resistant prostate cancer (CRPC) tissues

To identify and discover new kinase targets in metastatic CRPC, we analyzed 16 metastatic CRPC samples from 13 different patients obtained at rapid autopsy (*15*) by quantitative label-free phospho-tyrosine mass spectrometry (**Fig. 1**). These included 8 anatomically unique sites as well as 2 or 3 distinct sites from 3 separate patients (**Fig. 1**). Each sample contained greater than 50% tumor content as determined by histological analyses. We also analyzed 1 benign prostatic hyperplasia (BPH), 6 treatment naïve matched benign and cancerous prostates, and metastatic or subcutaneous xenograft tumors derived from the androgen insensitive 22Rv1 and androgen sensitive LNCaP cell lines (**Data file S1**)

(*8*). From three separate phospho-tyrosine enrichment preparations and mass spectrometry analyses, we identified 297 unique phospho-peptides corresponding to 185 unique proteins (**Data file S2**).

To compare different models and stages of prostate cancer, we included cell line-derived xenografts, treatment naïve primary prostate benign and cancerous tissues, and metastatic CRPC in a single phospho-tyrosine enrichment preparation. Unsupervised hierarchal clustering revealed three separate clusters. In particular, the cell line-derived xenografts grouped distinctly from any of the primary tissues independent of tumor location or treatment modality (**Fig. 2A**). Unsupervised hierarchal clustering also did not distinguish between the patient matched benign or cancerous prostates indicating that tyrosine phosphorylation remains relatively unchanged in treatment naïve benign or cancerous prostates (**Fig. 2A, fig. S1, A and B**). This indicates that the prostate cell line-derived xenografts evaluated indeed have different phosphorylation patterns compared to primary tissues. Supporting this notion, gene expression studies in small-cell lung cancer (SCLC) also identified primary tumor-specific signatures that were lost upon transitioning to cell culture (*16*) and proteomic analyses in colorectal cancer suggests that xenograft tumors are dramatically different than their cell line counterparts (*17*). This would suggest that validation or evaluation of metastatic CRPC tissues may be needed as a crucial step prior to moving forward with potential new therapeutic treatments.

Phosphoproteomic profiling and kinase/substrate enrichment analyses identifies several druggable kinase targets and pathways in metastatic CRPC lesions

Most patients with metastatic CRPC present with metastases at multiple sites creating a therapeutic dilemma (*15*). We set out to examine heterogeneity in a cohort of metastatic CRPC patients including those with multiple, anatomically distinct metastatic sites for activated kinase targets. Several metastatic CRPC patients that we evaluated contained similar anatomic sites of involvement including tumors in the liver, lung, dura, and distant lymph nodes. Unsupervised hierarchal clustering of the tyrosine phosphorylation patterns of 10 metastatic lesions, including 2 patients for which we had 2 independent metastatic lesions available, grouped these samples by patient rather than by metastatic site (**Fig. 2B**).

Phospho-tyrosine peptide identification and subsequent kinase/substrate relationship

analyses, which predicts kinase activity based on phospho-peptide motifs (*8*), identified several kinases and phosphatases (TYK2 Y^{292}, PTK2B Y^{579}, MAPK1/3 $Y^{187/204}$, DDR1 Y^{796}, the JAK2/SRC kinase target STAT3 Y^{705}, and PTPN11 $Y^{62/63}$) as well as putative upstream kinases and phosphatases (ALK, EGFR, PTK6, SRC, and PTPN2) in the metastatic CRPC samples (f**ig. S1, A-C, Data files S3-6**). These identifications are of great value as there are FDA approved or late stage clinical trial kinase inhibitors available for other diseases targeting SRC (dasatinib/bosutinib/ponatinib) (*18-20*), EGFR (erlotinib) (*21*), ALK (crizotinib) (*22*), the MAPK1/3 upstream pathway kinase MEK (trametinib) (*23*), or the STAT3 upstream kinase JAK2 (ruxolitinib) (*24*). Western blot analyses from 5 different patients confirmed the activation states of some of these kinases and also revealed interpatient heterogeneity as each patient evaluated displayed a unique phospho-pattern (**Fig. 2C**).

Large scale analyses of kinase activation patterns reveals intrapatient similarity across multiple, anatomically distinct metastases

As phospho-proteomic analyses revealed similar phospho-peptide patterns between anatomically distinct metastatic lesions from the same CRPC patient, we proceeded to examine the significance of this finding in a larger, independent set of patients. We evaluated 28 distinct metastatic lesions from 7 different CRPC patients (**fig. S2**). Western blot analysis of phospho-proteins identified by mass spectrometry and kinase/substrate enrichment analysis or the activated states of receptor tyrosine kinase (RTK) targets (EGFR Y^{1173}, ERBB2 Y^{1221}, and MET Y^{1234}) for which there are clinical inhibitors available confirmed our initial observation of intrapatient similarities (**Fig. 3, fig. S3, A-C**). Comparisons of different patients revealed dramatically different kinase activation patterns. This ranged from SRC Y^{419}, STAT3 Y^{705}, MAPK1/3 $T^{185/202}$/$Y^{187/204}$, and AKT S^{473}, activated upon PTEN loss in the majority of prostate cancers, for patient RA43 to only STAT3 Y^{705} for patient RA55 (**Fig. 3**). These unique phospho-patterns suggest that shared kinase activities exist in metastatic CRPC lesions isolated from the same patient.

To determine if this pattern of intrapatient similarity across metastases remains consistent with a larger set of other RTK and intracellular kinases, we evaluated 5 previously analyzed sets of patient metastases using RTK and phospho-kinase arrays from R&D

Systems. Three or 4 anatomically distinct metastatic lesions from each patient were analyzed and the patterns were found to be highly similar within a patient's set of metastatic lesions (**Fig 4A)**. Patient-specific patterns included the tyrosine phosphorylation of ALK, RYK, and the activation site of AKT T^{308} in patient RA37 as well as HCK Y^{411} from patient RA56 (**Fig. 4A**). Quantitation of these arrays revealed intrapatient similarities for 9 phospho- and total proteins (**Fig. 4B**). Four out of 5 patients evaluated displayed STAT2 activation (STAT2 Y^{689}) and β-catenin expression (**Fig. 4, A and B**). One protein found to be highly expressed in all patients evaluated was heat shock protein 60 (HSP60) (**Fig. 4, A and B**). This protein has been correlated to late stage, high Gleason score prostate cancer patients but the precise functional role in metastatic CRPC is currently not known (*25*). Principal component analysis (PCA) of the kinases and proteins with detectable phosphorylation or expression (n=11) confirmed intrapatient similarity (**Fig. 4C, fig. S4**). Plotting each of the 18 metastatic lesions on the first three principal components (77% of the total variance) demonstrated that each patient's lesions clustered together, regardless of the anatomical site of origin.

Stratification of metastatic CRPC patients' kinase activation patterns suggests that simultaneous targeting of SRC and MEK kinases may be of potential therapeutic value

Development of metastatic CRPC usually occurs after resistance to androgen blockade therapy and these patients ultimately succumb to their metastases. Treatment modalities may influence hormone escape and understanding the mechanisms of castration resistance may lead to better combination therapies (*26*). To determine new therapies for metastatic CRPC, we have to evaluate activated states of actionable, targetable proteins concomitant with new approaches and stratification methods so that new therapies can be properly evaluated for treatment of metastatic CRPC. All 16 individual metastatic CRPC lesions submitted for phospho-proteomics were evaluated to predict potential kinase inhibitor combinations. We pooled kinases that were identified from mass spectrometry, western blot, and predicted kinase/substrate relationships and revealed a wide range of predicted kinase activities across the patient samples (**Table 1**). Mapping clinically available inhibitors to these kinases revealed 11 different tyrosine kinase inhibitor combinations with overlap between 4 sets of inhibitor combinations (**Table 1**). Of note the SRC inhibitor, dasatinib, or the MEK inhibitor, trametinib, were predicted therapeutic strategies in 14/16 (87.5%) or

13/16 (81.2%) patients, respectively. If we consider combination therapy, 11/16 (68.8%) patients were predicted who might benefit from both dasatinib and trametinib inhibitors. There are no current clinical trials in prostate cancer evaluating the efficacy of SRC and MEK combination therapy, while there is one active Phase II study measuring the efficacy of this combination for metastatic breast cancer (ClinicalTrials.gov identifier: NCT00780676). While these predictions provide potential kinase inhibitor combinations, it confirms the complexity observed across patient samples and suggests that individualized therapy is important for clinical treatment decisions. Further, the kinases identified here may help to molecularly stratify these patients and direct the proper course of treatment.

Phosphorylation of neuronal RTKs RET and RYK in metastatic CRPC lesions with a small cell neuroendocrine carcinoma (SCNC) phenotype

Small cell neuroendocrine carcinoma (SCNC) of the prostate is a rare form of prostate cancer for which there are no effective treatments (*27*). Further evaluation of the phospho-RTK arrays revealed tyrosine phosphorylation of RET in patient RA33 (**Fig. 4A**). This is notable as this RTK is expressed in neuronal cell types suggesting this patient may have a neuroendocrine-like metastatic phenotype. Indeed histological analyses of patient RA33 confirmed that metastatic lesions have the classic histology of a SCNC as evidenced by a diffuse, solid growth pattern which is different from the conventional prostatic adenocarcinoma that shows glandular formation (f**ig. S5C, dashed circle**). In addition, the metastatic cells also demonstrate typical nuclear morphology of SCNC including a darkly stained nucleus with a homogeneous chromatin pattern, high nuclear/cytoplasmic (N/C) ratio, lack of nucleoli, and frequent mitotic figures (f**ig. S5B, arrows**). These characteristics are in sharp contrast to the nuclear morphology of adenocarcinoma cells that have open and vesicular chromatin patterns and prominent nuclei (f**ig. S5C, arrow**). This data suggests that the molecular phenotyping of SCNC, as indicated by phospho-RET activity, may drive new therapeutic strategies for this rarer subtype of prostate cancer.

DISCUSSION

From our study, we were able to measure protein phosphorylation in 41 metastatic CRPC samples from 17 patients including 16 samples designated for phospho-tyrosine

enrichment and mass spectrometry. Our phospho-kinase profiling and evaluation of active kinases suggests that kinase activity patterns are patient-specific and are maintained across multiple metastatic lesions within the same patient. This data supports previous studies suggesting that metastatic disease arises from a single precursor cancer cell or focal mass located at the primary tumor site (*6, 12-14*). Our findings add actionable information to this perspective. Kinase inhibitor treatment regimes guided by the biopsy of a single accessible metastatic lesion may be sufficient to predict the responses of multiple sites leading to a more efficacious use of single agents or multi-drug combinations.

The majority of patients with progressive prostate cancer develop metastatic castration-resistance. Treatment options in this stage had previously been limited to cytotoxic chemotherapies such as mitoxantrone (*28*) and docetaxel (*29*) for improved quality of life and modest survival benefits. However, several new therapeutic agents have been approved in the last three years for advanced prostate cancer including sipuleucel-T as autologous cellular immunotherapy (*30*), abiraterone acetate as a small molecule inhibitor of intra-tumoral androgen biosynthesis (*31*), and enzalutamide as a second-generation androgen receptor antagonist (*32*). In addition, a phase 1-2 clinical study investigating the combination of docetaxel with dasatinib (*33*), a SRC family kinase inhibitor, showed a high objective response rate of 60% in CRPC patients with this treatment regimen. Declines were also seen in PSA and bone markers with 30% of patients demonstrating resolution of at least one bone lesion on bone scan. Recent phase 2 clinical data also demonstrated notable activity of cabozantinib (XL-184), a MET and VEGFR2 inhibitor, in patients with CRPC (*34*). Despite an objective response rate of only 5% at 12 weeks, the disease control rate was 80%. 72% of patients achieved measurable regression in soft tissue lesions and 68% showed improvement on bone scans during the course of treatment. In parallel with the radiographic findings, patients reported a 67% diminution of bone pain and 56% reduction in the use of narcotic analgesics.

Development of new targeted therapies in metastatic CRPC is promising but also presents a number of clinical questions. Major challenges are to effectively stratify patients that will benefit from selected treatments and to recognize context-specific molecular targets. One approach to addressing these issues is the serial sampling and molecular characterization of malignant tissue from patients during the course of their disease. The increasing

availability of high-throughput tools have enabled the genomic and transcriptomic profiling of large numbers of clinical carcinoma samples of different subtypes (*4, 35, 36*). Phospho-proteomic technology, particularly mass spectroscopy-based proteomics, is also rapidly advancing and has recently been applied to the elucidation of tyrosine kinase-driven pathways in cell lines (*37-40*) or the discovery of activated kinases that may be useful for therapy in human cancers (*41, 42*).

Interestingly, very few patient sets were positive for the activated states of EGFR, ERBB2, or MET, although they were detected in prostate cancer cell lines. Drugs targeting EGFR and ERBB2 did not produce significant results in CRPC patients (*43, 44*), however, the MET agent cabozantinib has shown promise in the clinic (*34*). This is in contrast to our observation that MET activity is not detected in our analyzed metastatic CRPC tissues. One explanation is that our sampling of metastatic CRPC tissues is too small or that MET activity was lost prior to tissue collection and we were not able to detect it. Two other possibilities are that cabozantinib activity in metastatic CRPC is not targeted towards epithelial MET but rather to MET expressed in osteoblasts or other mesenchymal cells in the bone microenvironment (*34*) or cabozantinib is inhibiting another tyrosine kinase such as VEGFR2 or RET (*45*). While we did not evaluate VEGFR2 activity, we did observe RET activity in SCNC suggesting this kinase may be potentially targeted by cabozantinib in metastatic CRPC patients and should be evaluated further.

Rapid autopsy programs have paved the way for studies in genomic mutations, copy number alterations, and splicing variants from metastatic tissues that are otherwise difficult to obtain (*4, 6, 15, 46, 47*). Although we evaluated many soft tissue metastatic lesions, we were only able to evaluate 5 bone metastases. While bone metastases are evident in over 90% of metastatic CRPC patients (*15*), metastatic bone tumors are hard to study due to the lack of quality material available for analysis as tumor material is lodged into hard, calcified bone. This is also especially difficult considering the large amount of material required for phospho-proteomic preparations. A potential outcome could be that kinase patterns are principally determined by site of metastasis due to signals initiated by the surrounding local microenvironment creating a pre-metastatic niche (*48*). Tissue-specific kinase activation patterns were not observed in our study but further evaluation of bone metastases in patients also harboring soft tissue metastases will be necessary to extend these findings.

MATERIALS AND METHODS

Tissue culture of prostate cancer cell lines and derivation of xenograft tumors. 22Rv1 cells were grown in RPMI medium supplemented with L-glutamine, fetal bovine serum, and non-essential amino acids (NEAA). LNCaP, DU145, and C4-2 cells were grown in DMEM medium supplemented with L-glutamine, fetal bovine serum, and NEAA. 30 15-cm plates were collected from each cell line and treated with 2mM Vanadate for 30 minutes. Cells were subsequently lysed in 9M Urea lysis buffer and used for phospho-proteomic analysis.

To generate metastatic tumors, 1 x 10^5 22Rv1 cells were injected intracardially as previously described and dissemination was monitored using bioluminescence imaging (*49*). After 8 weeks, tumors were extracted from the metastatic locations including the liver and lymph nodes in the mesenteric and lung regions. Also, to evaluate primary tumor growth, 1 x 10^6 LNCaP cells were injected subcutaneously and excised once they reached DLAM limits.

Acquisition of clinically matched benign and cancerous primary prostate tissues and metastatic castration resistant prostate cancer (CRPC) samples. Patient samples were obtained from the UCLA Translational Pathology Core Laboratory (TPCL), which is authorized by the UCLA Institutional Review Board (IRB) to distribute anonymized tissues to researchers as described previously (*50-52*). Cancer and benign areas were clearly marked on the frozen sections slides and prostate tissue containing the cancer region was separated from the benign area prior to collecting for phospho-proteomic analyses.

The Rapid Autopsy program at the University of Michigan has been previously described (*11, 47*). Frozen tissues from the Rapid Autopsy program were sent overnight on dry ice for phospho-tyrosine peptide analysis. Sections were stained with hematoxylin and eosin for representative histology.

Quantitative analysis of phosphotyrosine peptides by mass spectrometry. Tissue lysis was performed as previously described (*8*). Briefly, greater than 300 mg of frozen tumor mass was homogenized and sonicated in urea lysis buffer (20 mM HEPES pH 8.0, 9 M urea, 2.5 mM sodium pyrophosphate, 1.0 mM beta-glycerophosphate, 1% N-octyl glycoside, 2 mM sodium orthovanadate). Total protein was measured using the BCA Protein Assay Kit

(Thermo Scientific/Pierce) and 25 mg of total protein was used for phospho-proteomic analysis. The remaining protein lysate was frozen for subsequent western blot analyses.

Phospho-tyrosine peptide enrichment and liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis was performed as previously described (*8, 37, 53*). Phospho-peptides were identified using the Proteome Discoverer software (version 1.4.0.88, Thermo Fisher Scientific). MS/MS fragmentation spectra were searched using SEQUEST against the Uniprot human reference proteome database with canonical and isoform sequences (downloaded January 2012 from uniprot.org). Search parameters included carbamidomethyl cysteine (*C) as a static modification. Dynamic modifications included phosphorylated tyrosine, serine, or threonine (pY, pS, pT, respectively) and oxidized methionine (*M). The Percolator node of Protein Discoverer was used to calculate false-discovery rate (FDR) thresholds and the FDR for the datasets was adjusted to 1% (version 1.17, Thermo Scientific). The Percolator algorithm uses a target-decoy database search strategy and discriminates true and false identifications with a support vector machine (*54*). The PhosphoRS 2.0 node was used to more accurately localize the phosphate on the peptide (*55*). Only phospho-peptides with at least one phospho-tyrosine assignment with a reported probability above 20% were considered. MS2 spectra for all reported phosphopeptides are deposited to the ProteomeXchange Consortium with the dataset identifier PXD000238 (*56*).

Data analysis. Data analysis was performed as previously described (*8*). For clustering, we removed any peptides which had an ANOVA score greater than 0.2. Hierarchical clustering was performed using the Cluster program with the Pearson correlation and pairwise complete linkage analysis (*57*). Java TreeView was used to visualize clustering results (*58*). Quantitative data for each phosphopeptide can be found in **Data files S7-9**.

Prediction of kinase-substrate relationships and enrichment analysis of kinase activity. Predictions, enrichment, and permutation analyses have been previously described (*8*). Phospho-tyrosine peptides were ranked by the signal-to-noise ratio observed for a given perturbation (e.g. metastatic CRPC compared to benign prostate or benign prostatic hyperplasia). The enrichment scores for all putative upstream kinases are shown in **Data files S4-6**.

Western blot. For westerns, equal protein amounts of metastatic CRPC tissue urea lysates (20 or 30 μg) were used from tissues prepared as described previously (*8*). Antibodies were diluted as follows: AKT (1:1000, Santa Cruz), pAKT S^{473} (1:2000, Cell Signaling), EGFR (1:1000, Cell Signaling), pEGFR Y^{1173} (1:1000, Cell Signaling), STAT3 (1:1000, Cell Signaling), pSTAT3 Y^{705} (1:2000, Cell Signaling), JAK2 (1:1000, Cell Signaling), pJAK2 $Y^{1007/1008}$ (1:500, Cell Signaling), MAPK1/3 (1:1000, Cell Signaling), MAPK1/3 $T^{185/202}/Y^{187/204}$ (1:2000, Cell Signaling), SRC (1:1000, Millipore), pSRC Y^{419} (1:1000, Cell Signaling), ERBB2 (1:1000, Cell Signaling), pERBB2 $Y^{1221/1222}$ (1:1000, Cell Signaling), MET (1:1000, Cell Signaling), pMET Y^{1234} (1:1000, Cell Signaling). ECL substrate (Millipore) was used for detection and development on GE/Amersham film.

Phospho-receptor tyrosine kinase (RTK) and phospho-kinase arrays. Human Phospho-RTK (R&D Sytems) and Phospho-Kinase (R&D Systems) arrays were utilized according to manufacturer's instructions. Briefly, 300 ug of 9M urea lysate for each metastatic sample was diluted in the kit-specific dilution buffer to a final concentration of 0.85M urea and incubated with blocked membranes overnight. The membranes were washed and exposed to chemiluminescent reagent and developed on GE/Amersham film.

Principal Component Analysis. Each antibody-related spot on the Phospho-RTK and Phospho-Kinase arrays was quantified using Image J. After background subtraction, the duplicate spots for each antibody were averaged, and antibodies with negligible signal were removed. The data was unit normalized, and principal components were calculated in R.

SUPPLEMENTARY MATERIALS

Fig. S1. Distinct clusters of phosphorylation between the cell line-derived xenografts and primary prostate tissues.

Fig. S2. Anatomical location and histology of 7 CRPC patients

Fig. S3. Evaluation of phosphorylated kinases in prostate cancer cell lines.

Fig. S4. Principal component analysis of phospho-kinase arrays.

Fig. S5. Tyrosine phosphorylation of RET in small cell neuroendocrine carcinoma (SCNC).

APPENDIX A OF THIS DOCUMENT COMPRISES DATA FILES S1-S9

Data file S1. Patient list of primary prostate and metastatic CRPC tissues

Data file S2. Phospho-peptides identified across all 3 mass spectrometry preparations

Data file S3. Phospho-peptide and pathway analysis in metastatic CRPC samples

Data file S4. Inference of kinase activity - Batch #1

Data file S5. Inference of kinase activity - Batch #2

Data file S6. Inference of kinase activity - Batch #3

Data file S7. Quantitative MS data for each phospho-peptide identified in batch #1

Data file S8. Quantitative MS data for each phospho-peptide identified in batch #2

Data file S9. Quantitative MS data for each phospho-peptide identified in batch #3

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Table 1: Kinase and Inhibitor Stratification of Metastatic CRPC Patients

Patient Number and Metastatic Location	Identified Kinases via MS and Western Blot plus Inferred Kinases via Kinase/Substrate Relationships[1]	Potential Clinical Inhibitors				
		Dasatinib[2]	Erlotinib[3]	Crizotinib[4]	Ruxolitinib[5]	Trametinib[6]
RA06 Liver	EPHA3-7, SRC, PDGFR	X				
RA13 Mediastinal LN	ALK, FLT3/CSF1R/KIT, INSR, MAPK1, MAP3K2, PTK6, SRC	X		X		X
RA14 Liver	EGFR, MAPK1, MAP3K2, PTK6	X	X			X
RA30 Mediastinal LN	ALK, FLT3/CSF1R/KIT, MAP3K2, PTK6, SRC	X		X		X
RA40 Prostate	EGFR, MAPK1/3, MAP2K2, MAP3K2, PTK6, SRC	X	X			X
RA41 Dura	FLT3/CSF1R/KIT, MAPK1/3, SRC	X				X

RA43 Peritoneal and Right Lung	ALK, EGFR, EPHA3-7, MAPK1/3, PTK6, SRC	X	X	X		X
RA43 Periaortic LN	MAPK1/3, SRC	X				X
RA43 Right Lung	EGFR, FLT3/CSF1R/KIT, MAPK1/3, MAP2K2	X	X			X
RA45 Liver	ALK, MAP3K2			X		X
RA50 Periaortic LN	MAPK1/3, MAP3K2					X
RA53 Left Femur and Left Lung	ALK, EPHA3-7, JAK2, MAPK1/3, PDGFR, PTK6, SRC	X		X	X	X
RA55 Liver	ALK, EGFR, EPHA3, MAPK1/3, MAP2K2, MAP3K2, PTK6	X	X	X		X
RA55 Dura	EGFR, PTK6	X	X			
RA56 Perihilar LN	EGFR, HCK, TYK2	X	X		X	
RA57 Liver	EPHA7, MAP3K2, TYK2	X			X	X

[1]Kinases corresponding to identified phospho-peptides observed as >2-fold over benign tissues, via western blotting, or kinase/substrate relationships (<0.1 p-value) as shown in Supplementary Tables 4-6. [2]SRC family kinase, KIT, PDGFR, EPHA receptor inhibitor. [3]EGFR inhibitor. [4]ALK inhibitor. [5]JAK2 inhibitor. [6]MEK inhibitor.

The foregoing description of one or more embodiments of the invention has been presented for the purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise form disclosed. Many modifications and variations are possible in light of the above teaching.

All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

CLAIMS

1. A method of obtaining information useful for the diagnosis, prognosis and/or selection of a therapeutic protocol of a person suffering from or suspected of suffering from a selected cancer, the method comprising:

observing the phosphorylation profile of one or more peptides disclosed herein; and

comparing the observed phosphorylation profile with a phosphorylation profiles observed:

in patients diagnosed with the cancer;

in patients having a defined cancer prognosis; and/or

in patients observed to be response or non-responsive to a selected therapeutic protocol or agent used to treat the cancer;

so that information useful for the diagnosis, prognosis and/or selection of a therapeutic protocol of a person suffering from or suspected of suffering from the cancer is obtained.

2. The method of claim 1, wherein the method further comprises using the information to:

select a therapeutic agent; and

administering the agent to the patient.

3. The method of claim 2, wherein the therapeutic agent is a kinase inhibitor.

4. The method of claim 2, wherein the therapeutic agent inhibits the activity of c-Src tyrosine kinase, MEK-1 threonine and tyrosine kinase, or RET tyrosine kinase.

5. The method of claims 3-4, wherein a plurality of therapeutic agents are administered to the patient.

6. The method of claim 1, wherein the phosphorylation profiles of 2, 3, 4, or 5 peptides disclosed herein are observed.

7. The method of claim 1, wherein the cancer is prostate cancer.

8. The method of claim 6, wherein the prostate cancer is a castration resistant prostate cancer.

9. An isolated phosphopeptide disclosed herein.

10. A kit comprising a first antibody that binds a first phosphopeptide disclosed herein and second antibody that binds a second phosphopeptide disclosed herein.

ABSTRACT

We identified distinct phospho-peptide patterns in metastatic tissues compared to naive primary prostate tissue and prostate cancer cell line-derived xenografts. Evaluation of metastatic CRPC samples for tyrosine phosphorylation and upstream kinase targets revealed SRC, EGFR, RET, ALK, and MAPK1/3 and other activities while exhibiting intrapatient similarity and interpatient heterogeneity. This data provides evidence that individualized therapy targeting non-mutated kinases with clinical kinase inhibitors may be an effective strategy in the diagnosis, prognosis and treatment of metastatic CRPC.

APPENDICES

Aspects of this invention are described in supplementary data files S1-S9 provided in Appendix A. Aspects of this invention are also described in a poster format that is provided in Appendix B.

APPENDIX A

APPENDIX B

Figure 1

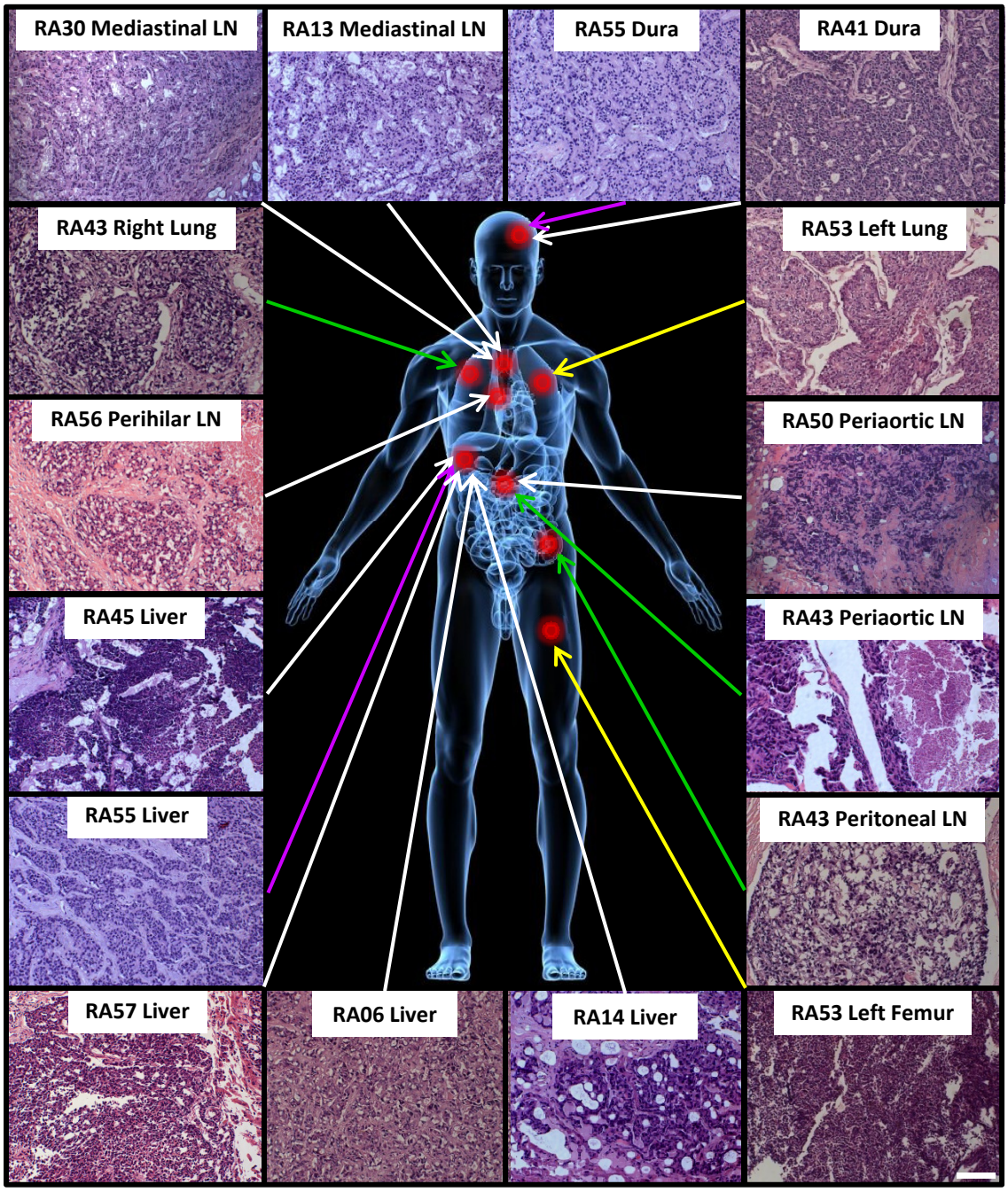

RA30 Mediastinal LN
RA13 Mediastinal LN
RA55 Dura
RA41 Dura
RA43 Right Lung
RA53 Left Lung
RA56 Perihilar LN
RA50 Periaortic LN
RA45 Liver
RA43 Periaortic LN
RA55 Liver
RA43 Peritoneal LN
RA57 Liver
RA06 Liver
RA14 Liver
RA53 Left Femur

Figure 2

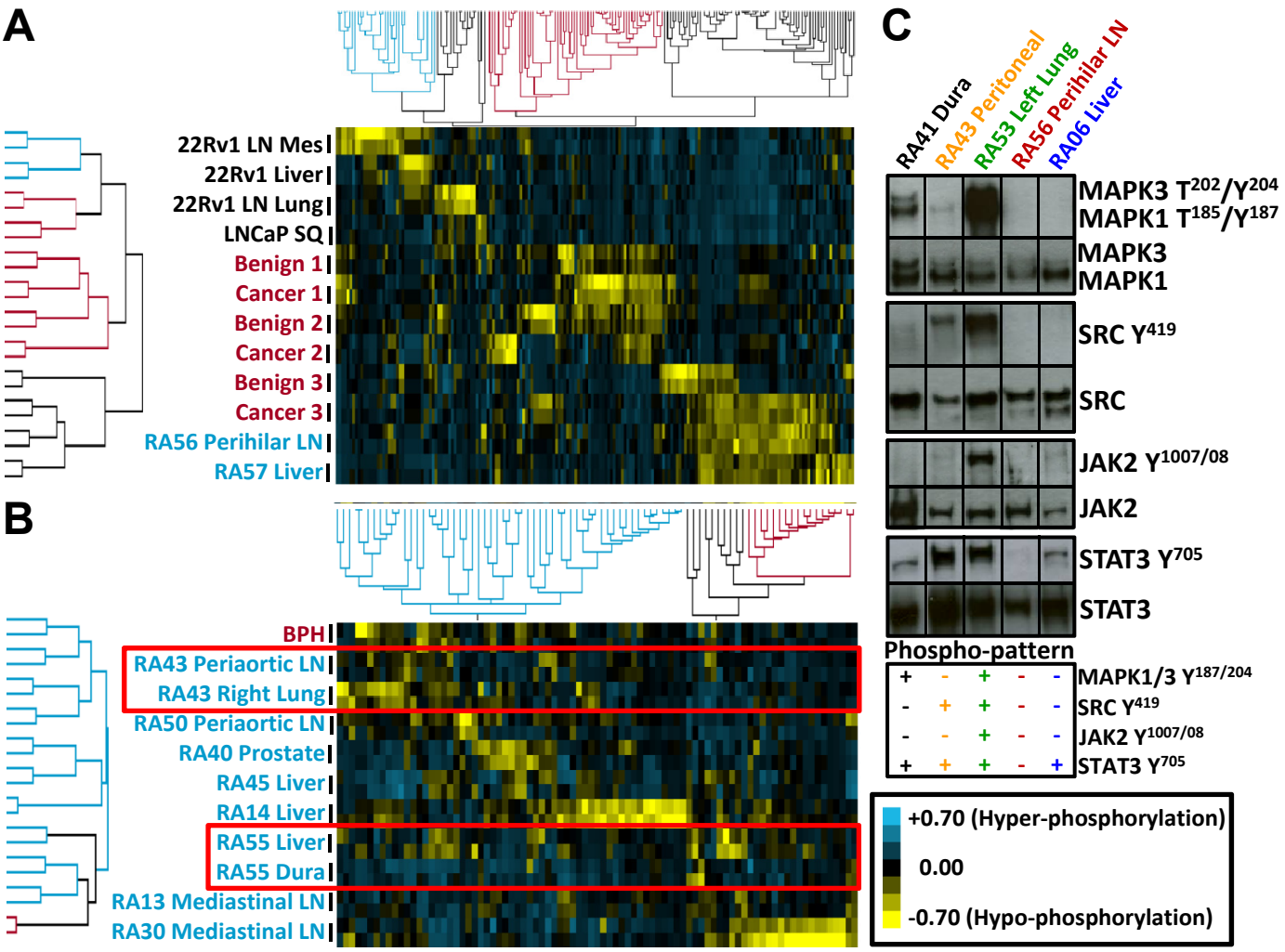

A
22Rv1 LN Mes
22Rv1 Liver
22Rv1 LN Lung
LNCaP SQ
Benign 1
Cancer 1
Benign 2
Cancer 2
Benign 3
Cancer 3
RA56 Perihilar LN
RA57 Liver
B
BPH
RA43 Periaortic LN
RA43 Right Lung
RA50 Periaortic LN
RA40 Prostate
RA45 Liver
RA14 Liver
RA55 Liver
RA55 Dura
RA13 Mediastinal LN
RA30 Mediastinal LN
C
RA41 Dura
RA43 Peritoneal
RA53 Left Lung
RA56 Perihilar LN
RA06 Liver
MAPK3 T202/Y204
MAPK1 T185/Y187
MAPK3
MAPK1
SRC Y419
SRC
JAK2 Y1007/08
JAK2
STAT3 Y705
STAT3
Phospho-pattern
+ - + - -
- + + - -
- - + - -
+ + + - +
MAPK1/3 Y187/204
SRC Y419
JAK2 Y1007/08
STAT3 Y705
+0.70 (Hyper-phosphorylation)
0.00
-0.70 (Hypo-phosphorylation)

Figure 3

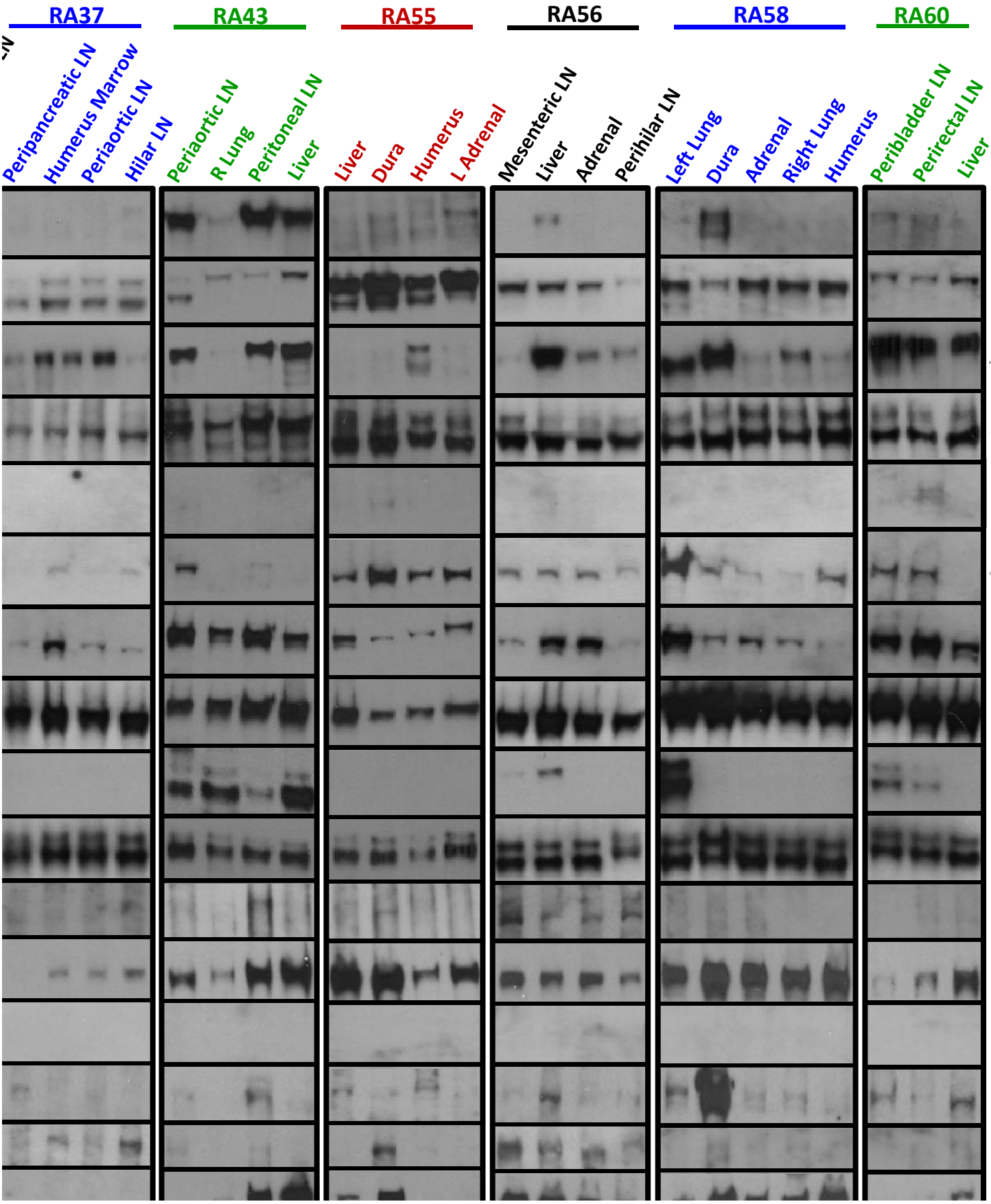
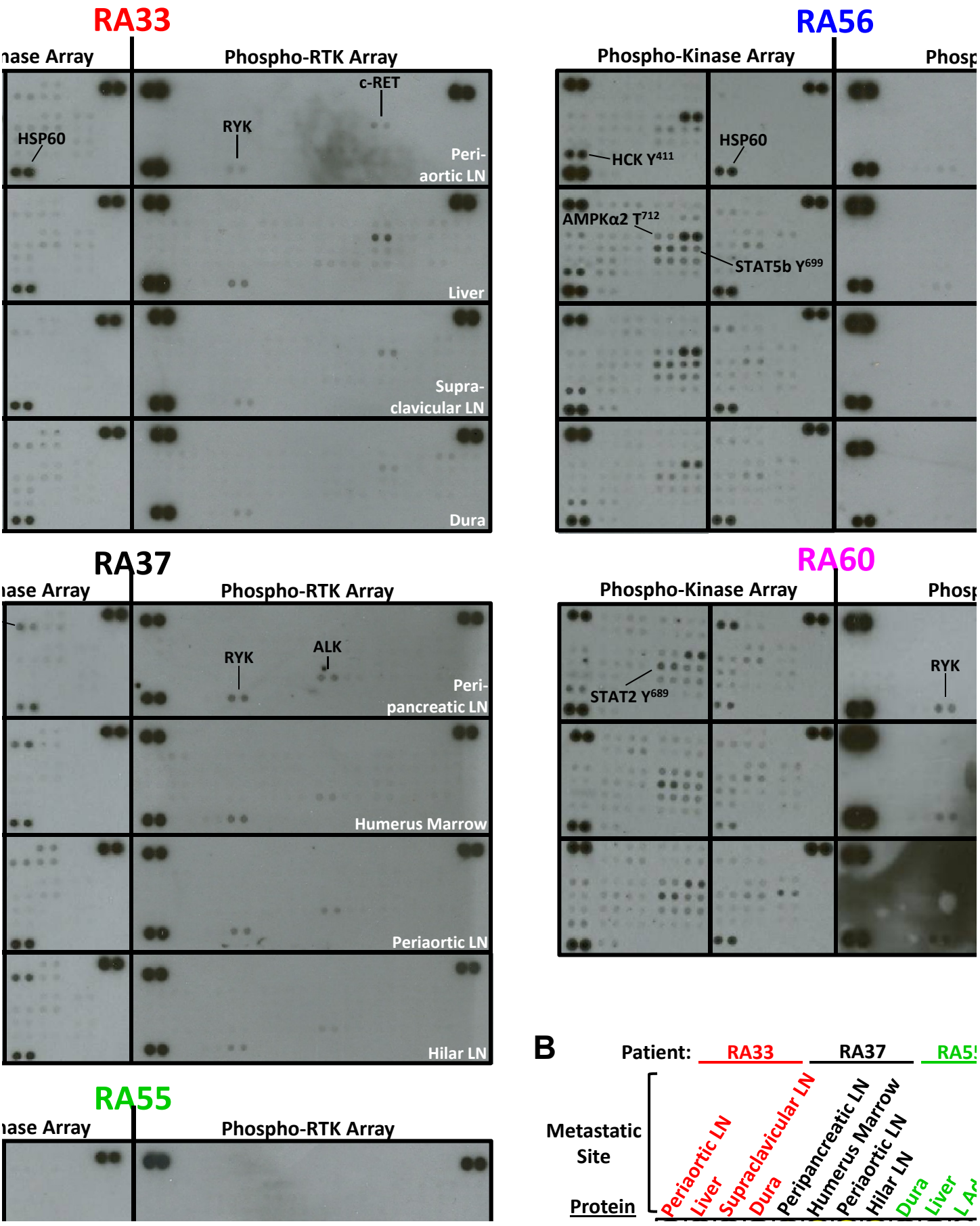
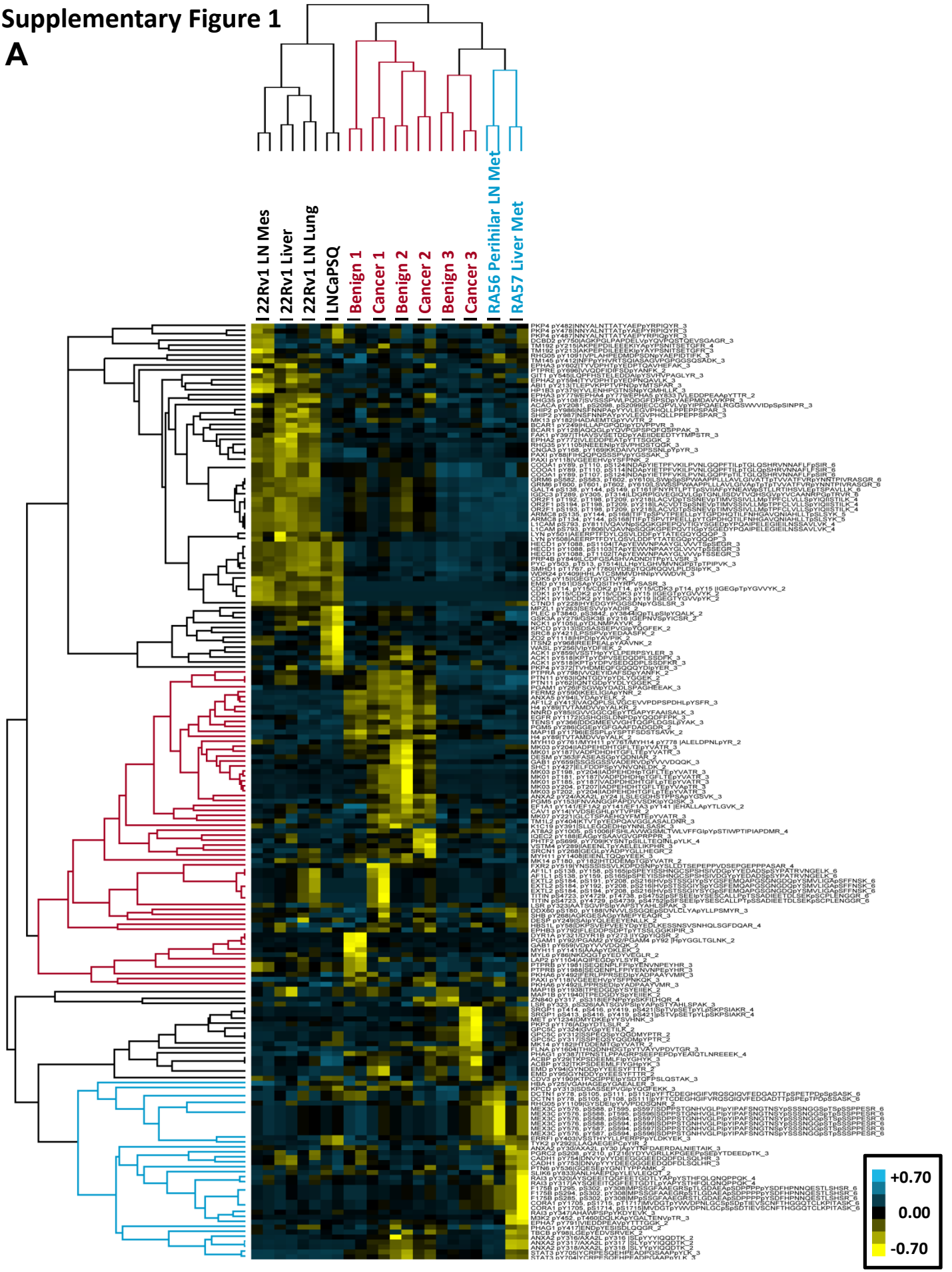

RA37
RA43
RA55
RA56
RA58
RA60
Peripancreatic LN
Humerus Marrow
Periaortic LN
Hilar LN
Periaortic LN
R Lung
Peritoneal LN
Liver
Liver
Dura
Humerus
L Adrenal
Mesenteric LN
Liver
Adrenal
Perihilar LN
Left Lung
Dura
Adrenal
Right Lung
Humerus
Peribladder LN
Perirectal LN
Liver

Figure 4


RA33
ıase Array
Phospho-RTK Array
HSP60
RYK
c-RET
Peri-aortic LN
Liver
Supra-clavicular LN
Dura
RA56
Phospho-Kinase Array
Phosp
HCK Y411
HSP60
AMPKα2 T712
STAT5b Y699
RA37
ıase Array
Phospho-RTK Array
RYK
ALK
Peri-pancreatic LN
Humerus Marrow
Periaortic LN
Hilar LN
RA60
Phospho-Kinase Array
Phosp
STAT2 Y689
RYK
RA55
ıase Array
Phospho-RTK Array
B
Patient:
RA33
RA37
RA5
Metastatic Site
Protein
Periaortic LN
Liver
Supraclavicular LN
Dura
Peripancreatic LN
Humerus Marrow
Periaortic LN
Hilar LN
Dura
Liver

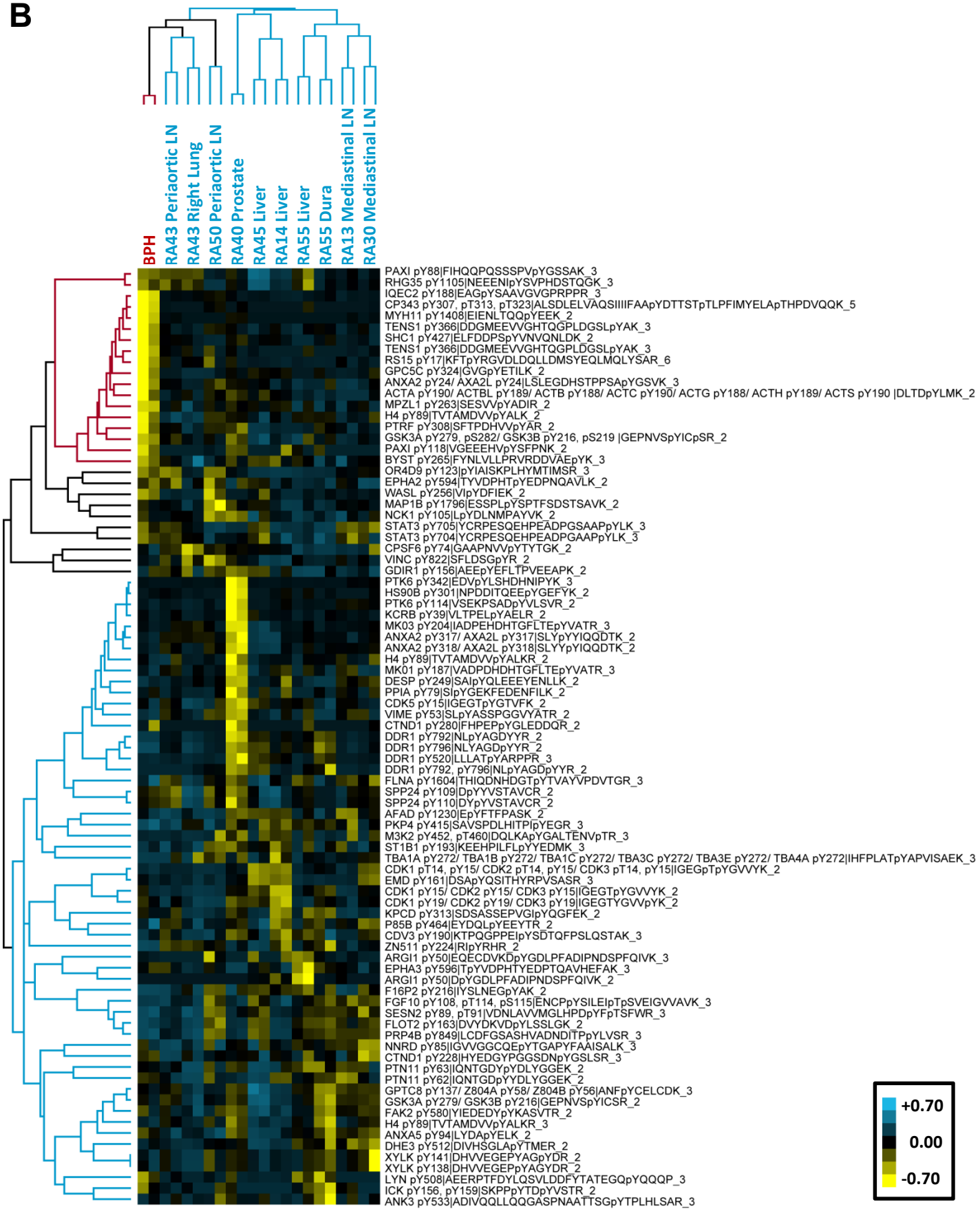
Supplementary Figure 1

A

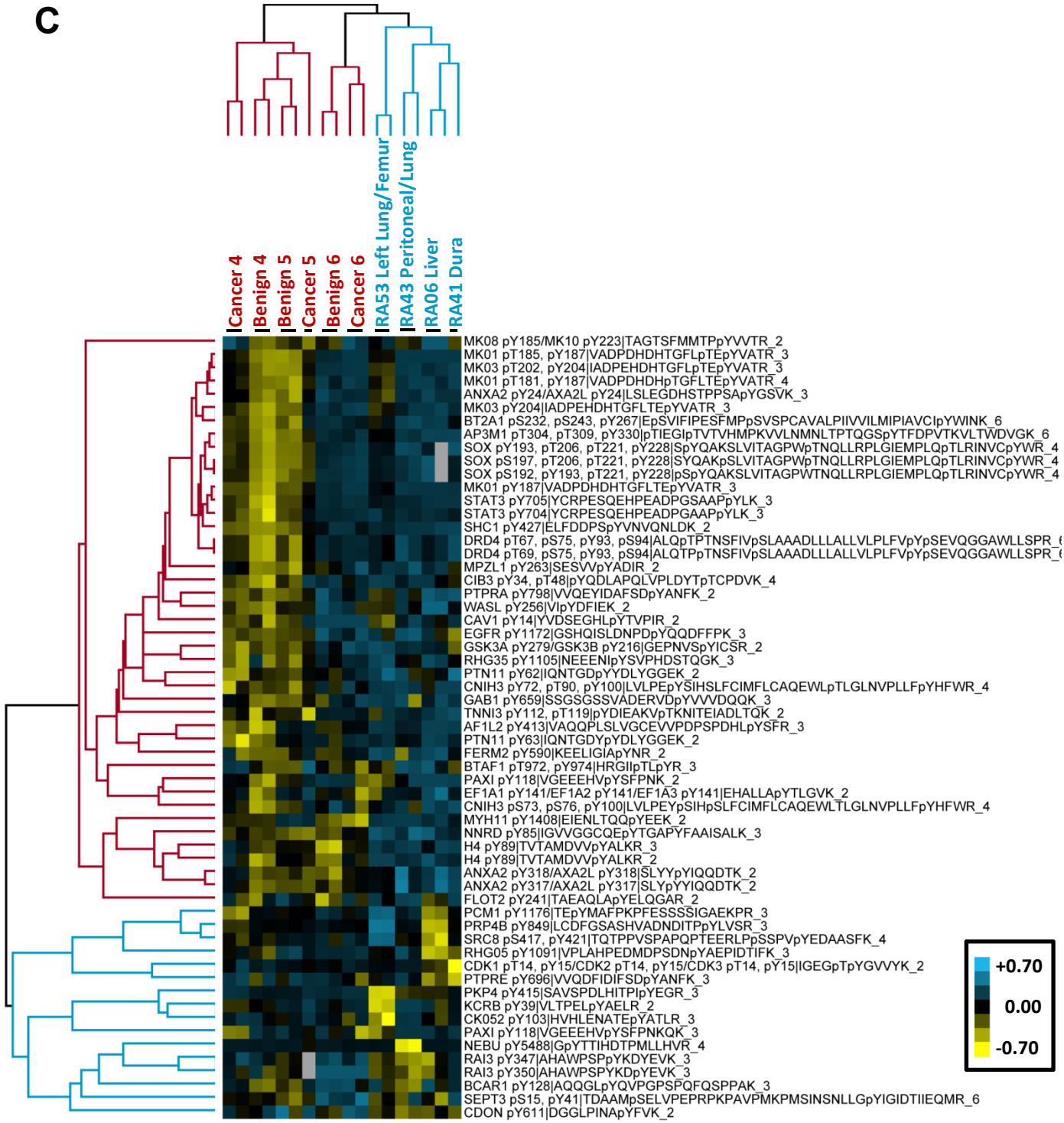


Supplementary Figure 1

B

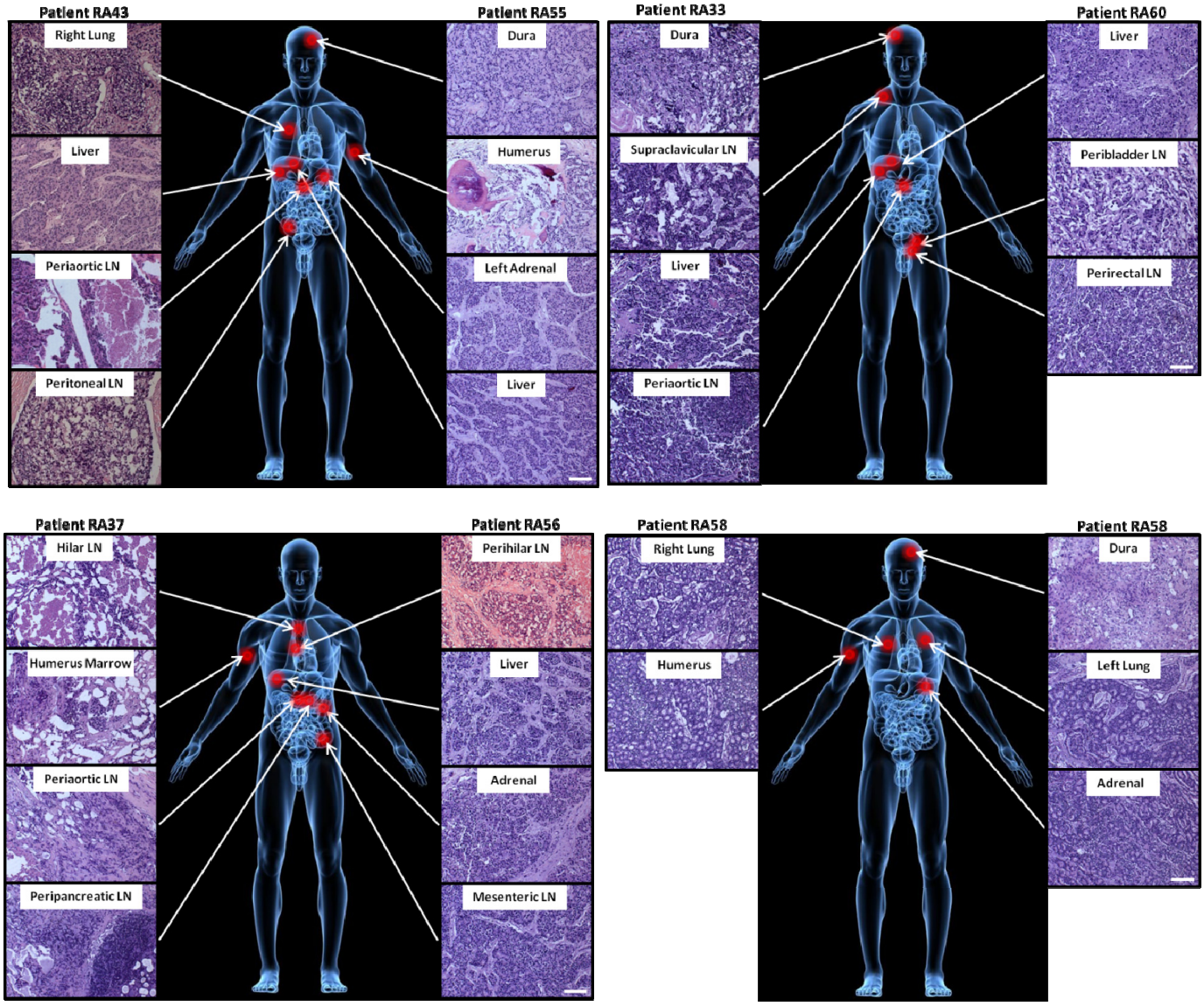


Supplementary Figure1



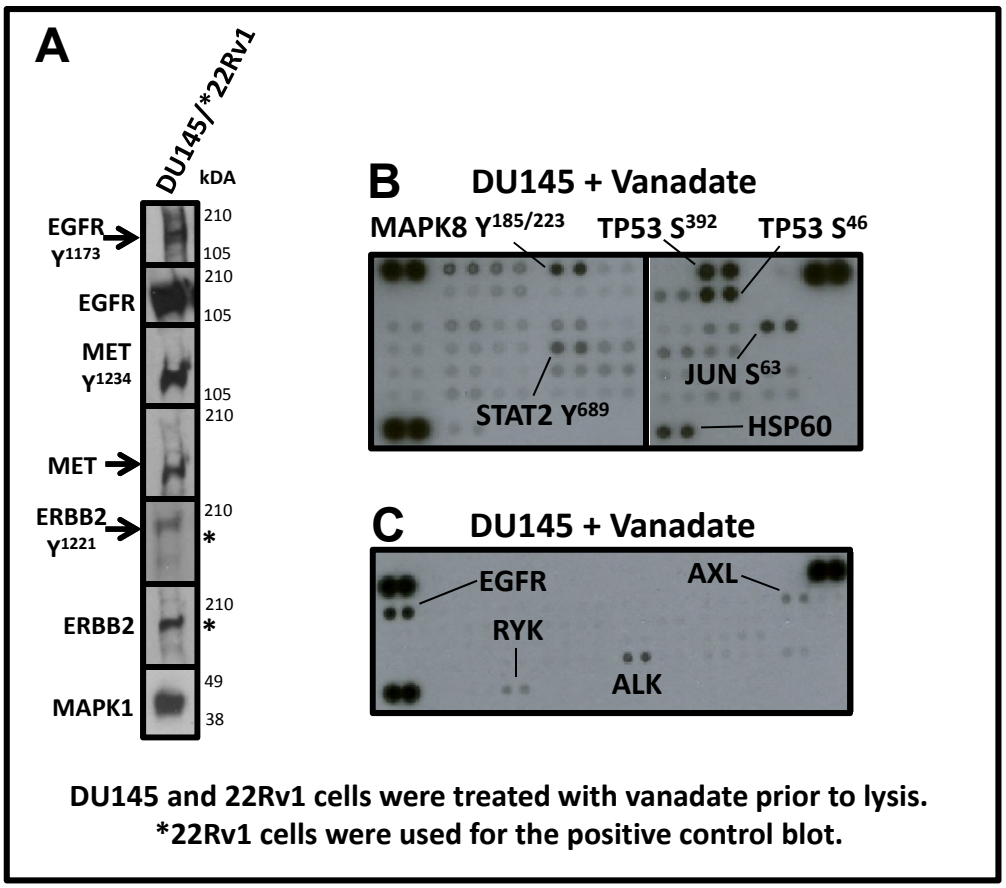
Supplementary Figure 1. Phosphoproteomic analyses exhibits distinct clusters of phosphorylation between the cell line-derived xenografts and primary prostate tissues. (A) Unsupervised heirarchal clustering does not group cell line-derived metastatic xenograft tumors with either organ confined or metastatic CRPC. Also, treatment naïve patient matched benign and cancerous prostates display indistinguishable phospho-peptide signatures. The phospho-profiling heatmap from Figure 2a with the protein and residue identities of the phosphorylation events are listed. (B) Unsupervised heirarchal clustering does not group by organ site of metastases but rather by intra-patient metastatic lesions. Benign prostatic hyperplasia (BPH) was used as the treatment naïve tissue for comparison. The phospho-profiling heatmap from Figure 2b with the protein and residue identities of the phosphorylation events are listed. (C) Unsupervised heirarchal clustering does not group organ confined prostate benign or cancerous prostates with metastatic CRPC. Also, treatment naïve patient matched benign and cancerous prostates display indistinguishable phospho-peptide signatures. The phospho-profiling heatmap from Batch 2 with the protein and residue identities of the phosphorylation events are listed. For all heatmaps, the labels are as follows: UniProt ID, phosphosite residue number, phospho-peptide (charge state of mass spectrometry ion). If the phospho-peptide has multiple identities, a slash separates each protein and phosphorylation residue number. The vertical line separates the proteins from the phospho-peptide.
Yellow=hyperphosphorylation, Blue=hypophosphorylation.

Supplementary Figure 2



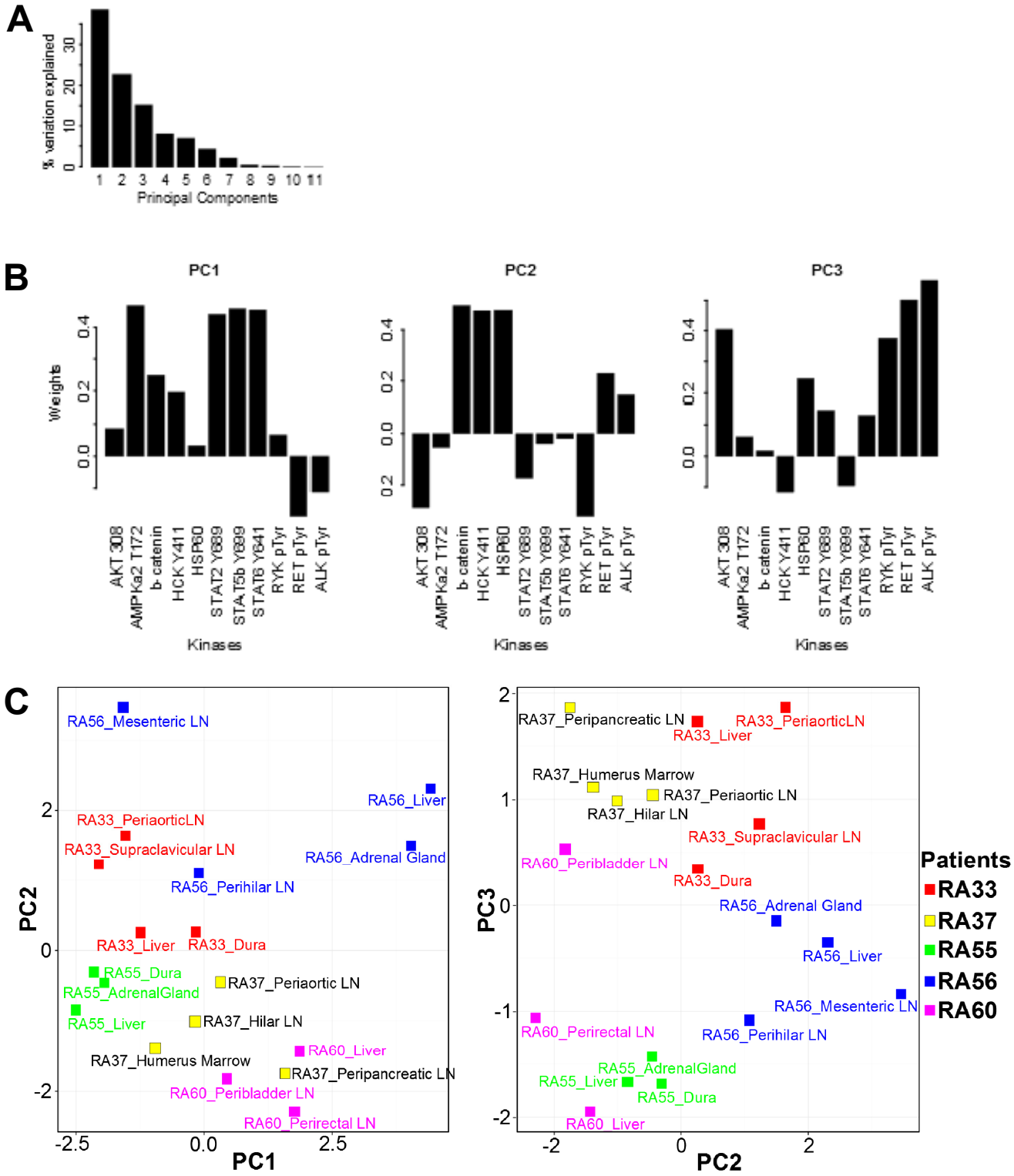
Supplementary Figure 2. Location and histological characterization of 7 patients with anatomically distinct metastatic castration resistant prostate cancer (CRPC) lesions. 7 separate patients' metastatic lesions are depicted with representative histology. These samples were used for western blot and phospho-RTK and phospho-kinase arrays. Red dots indicate the approximate location of the metastatic lesions analyzed. Tissues with greater than 50% tumor content were evaluated. Scale bar=50 μm.

Supplementary Figure 3



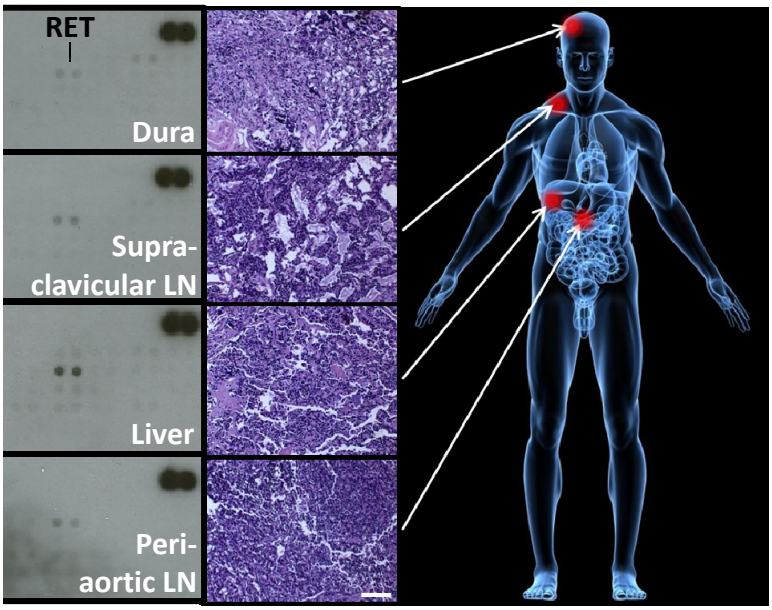
Supplementary Figure 3. Evaluation of receptor tyrosine kinase (RTK) EGFR, ERBB2, and MET and phospho-kinase and phospho-RTK arrays using positive control prostate cancer cell lines. Western blot analyses from DU145 or 22Rv1 cells treated with the phosphatase inhibitor, vanadate, were evaluated for the activated states of the receptor tyrosine kinases (RTKs) EGFR, ERBB2, and MET (A), phospho-kinase (B), or phospho-RTK arrays (C). DU145 or 22Rv1 (indicated by an asterisk next to the blot) cells were used as positive controls.

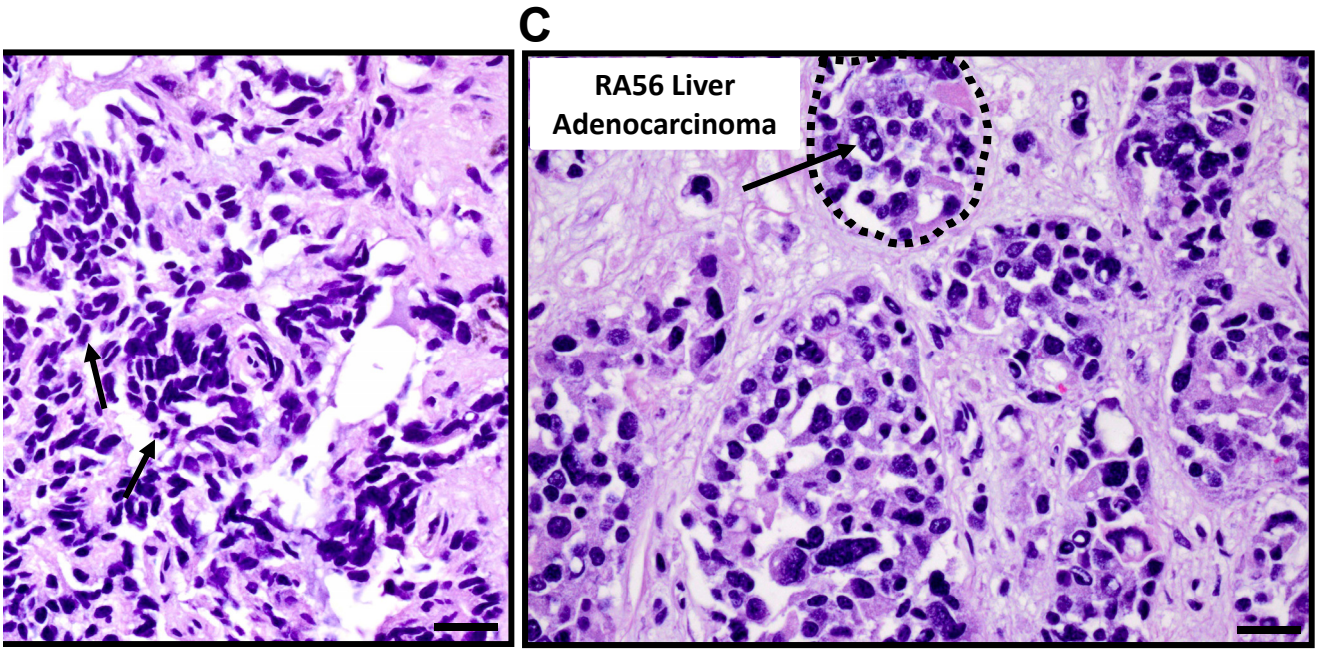
Supplementary Figure 4



Supplementary Figure 4. Principal Component Analysis of Phospho-Kinase arrays. Data from CRPC metastatic samples analyzed by phospho-kinase arrays was subjected to principal component analysis. After removal of antibodies with negligible signal, 11 antibodies remained: Akt T^{308}, AMPKa T^{172}, β-catenin, HCK Y^{411}, STAT2 Y^{689}, STAT5b Y^{699}, STAT6 Y^{641}, RYK phospho-tyrosine, RET phospho-tyrosine, and ALK phospho-tyrosine. (A) Schematic of the loadings vectors for the first three principal components. (B) The percentages listed for each principal component (PC) indicated the amount of variance explained by that PC. (C) Plots of the PCA for all 5 patients analyzed demonstrate intra-patient kinase expression similarity and individual differences.

Supplemental Figure 5





gure 5. Tyrosine phosphorylation of receptor tyrosine kinase (RTK) RET in small cell rcinoma (SCNC). (A) Analysis of patient RA33 using RTK arrays revealed the tyrosine neuronal tyrosine kinase RET. (B) Metastatic tumor cells in this patient demonstrate typical nuclear CNC including a darkly stained nucleus with a homogeneous chromatin pattern, high e (N/C) ratio, lack of nucleoli, and frequent mitotic figures (B, arrows). These characteristics are in e nuclear morphology of adenocarcinoma tumor cells (C) that have open and vesicular chromatin ent nuclei (C, arrow) and glandular formation (C, dashed circle). Scale bar=25 μm.

Phosphoproteomic Analysis of Metastatic Castration Resistant Prostate Cancer Reveals Distinct Subsets of Tyrosine Kinase Activities

Justin M. Drake, Nicholas A. Graham, John K. Lee, Tanya Stoyanova, Sudha Sud, Bjoern Titz, Jiaoti Huang, Kenneth J. Pienta, Thomas G. Graeber, Owen N. Witte

Abstract

Prostate cancer is the most commonly diagnosed and second leading cause of cancer related death in American men. Despite the numerous oncogenic alterations implicated in prostate cancer, dominant activating mutations or DNA amplification of tyrosine kinases are not readily observed. We demonstrate that castration resistant prostate cancer (CRPC) exhibits increased tyrosine phosphorylation, raising the question of whether enhanced tyrosine kinase activity is observed in this disease in the absence of specific tyrosine kinase mutations. With access to metastatic CRPC material from the University of Michigan's Rapid Autopsy Program, we have evaluated tyrosine kinase activity from lethal metastatic CRPC patients. Phosphotyrosine peptide enrichment and quantitative mass spectrometry identified diverse phosphorylation events in the metastatic tissues when compared to naive primary prostate tissue and prostate cancer xenografts. Kinase:substrate relationship analysis of the phosphopeptides also revealed SRC Y^{416} tyrosine kinase activation in the metastatic CRPC tissues. Western blot and tissue IHC revealed that distinct groups of metastatic tumors expressed specific activated tyrosine kinases, including SRC Y^{416}, JAK2 Y^{1007}, and ERK1/2 T^{202}/Y^{204}. In summary, the observation of enriched tyrosine kinase activities in subsets of metastatic CRPC tissues suggests that these patients may benefit from targeted therapies.

PROSTATE CANCER FOUNDATION

2013 PROUTS NECK 2.0 MEETING ON PROSTATE CANCER

Beyond AR: New Approaches to Treating Metastatic Prostate Cancer

June 20-23, 2013
Hyatt Regency
Lake Tahoe, Nevada

Program Committee

Ken Pienta, M.D. Chair (Johns Hopkins University)
John Isaacs, Ph.D. (Johns Hopkins University)
Phil Kantoff, M.D. (Dana-Farber Cancer Center)
Peter Nelson, M.D. (Fred Hutchinson Cancer Research Center)
Howard Soule, Ph.D. (Prostate Cancer Foundation)
Owen Witte, M.D. (UCLA)

Hyatt Regency Lake Tahoe
111 Country Club Drive
Incline Village, Nevada 89451
Phone: 775.832.1234

THURSDAY, JUNE 20, 2013

3:00 PM - 7:00 PM	Arrival/Check-In & Registration *Registration Desk*	*Regency Foyer*
5:00 PM - 6:00 PM	Reception	*Mike Milken Lakeshore Home*
6:00 PM - 7:30 PM	Dinner	*Mike Milken Lakeshore Home*

Session I: Introduction and Charge for Participants
Location: *Mike Milken Lakeshore Home*

7:30 PM - 8:00 PM	Welcome and Opening Remarks	**Ken Pienta, MD** **Howard Soule, PhD**
	History of Prouts Neck	**Donald Coffey, PhD**
8:00 PM - 9:00 PM	Global & Personal Perspectives	**Mike Milken**

FRIDAY, JUNE 21, 2013

Session II: Charge to Participants: The Big Questions
Location: *Regency Ballroom DEF*

7:00 AM - 7:45 AM	Breakfast	*Regency Ballroom DEF*
8:00 AM – 9:00 AM	The Big Questions	**Ken Pienta, MD**

Session III: Extrinsic (Microenvironment) Factors Modulating Prostate Cancer Therapy Resistance
Location: *Regency Ballroom DEF*

Session Chair:	**Ralph Buttyan, PhD**
9:00 AM - 9:10 AM	**Stromal Mediators of Resistance** **Peter Nelson, MD** Fred Hutchinson Cancer Research Center
9:10 AM - 9:30 AM	Discussion First Question: Mark Pomerantz

9:30 AM - 9:40 AM	**DNA Damage-Induced Signaling and the Survival of Minimal Residual Disease** **Michael Hemann, PhD** Massachusetts Institute of Technology
9:40 AM – 10:00 AM	Discussion First Question: Chad Brenner
10:00 AM - 10:10 AM	**Role of Epithelial to Mesenchymal Transition in Prostate Cancer Therapy Resistance** **Rob Reiter, MD** David Geffen School of Medicine at UCLA
10:10 AM - 10:30 AM	Discussion First Question: Jennifer Bishop
10:30 AM – 10:40 AM	Break *Regency Foyer*
10:40 AM - 10:50 AM	**A Strategy to Improve Cancer Therapy Models in Mice to Better Predict Outcomes of Patients with Metastatic Disease** **Robert S. Kerbel, PhD** University of Toronto
10:50 AM - 11:10 AM	Discussion First Question: John Isaacs
11:10 AM - 11:20 AM	**Stromal and Epithelial Mediators of Resistance to Immunotherapy for Prostate Cancer** **Charles Drake, MD, PhD** Johns Hopkins University
11:20 AM - 11:40 AM	Discussion First Question: Guneet Walia
11:40 AM -11:50 AM	**A Different Look at the Hallmarks of Cancer** **Yuzhuo Wang, PhD** The Vancouver Prostate Centre
11:50 AM - 12:10 PM	Discussion First Question: Yu Chen
12:10 PM -12:20 PM	**The Association Between the Stem Cell State and Prostate Cancer Resistance - Evaluating the Role of the Microenvironment and Treatment Effects in Promoting Lethal Disease** **Isla Garraway, MD, PhD** David Geffen School of Medicine at UCLA
12:20 PM - 12:40 PM	Discussion First Question: Chris Maher

12:40 PM - 1:40 PM	Lunch	*Water Garden I*
1:40 PM - 2:00 PM	Move to Session	

Session IV: Defeating Therapeutic Resistance
Location: *Regency Ballroom DEF*

Session Chair: **Mark Rubin, MD**

2:00 PM - 2:10PM
Introduction to Session
Mark Rubin, MD
Weill Cornell Medical College

2:10 PM - 2:20 PM
Co-Targeting Adaptive Stress Response Pathways to Manipulate Sensitivity of Cancer Cells to Therapy
Martin Gleave, MD
University of British Columbia

2:20 PM - 2:40 PM
Discussion
First Question: Joshua Lang

2:40 PM - 2:50 PM
Immune Checkpoint Blockade in Cancer Therapy: New Insights and Opportunities
James Allison, PhD
The University of Texas MD Anderson Cancer Center

2:50 PM - 3:10 PM
Discussion
First Question: Akash Patnaik

3:10 PM - 3:20 PM
Investigating Anti-CTLA-4 as Treatment for Prostate Cancer
Padmanee Sharma, MD, PhD
The University of Texas MD Anderson Cancer Center

3:20 PM - 3:40 PM
Discussion
First Question: Felix Feng

3:40 PM - 3:50 PM
Defining Kinase Targets in Advanced Prostate Cancer
Justin Drake, PhD
University of California, Los Angeles

3:50 PM - 4:10 PM
Discussion
First Question: Hannelore Heemers

4:10 PM - 4:20 PM
Implementing a Precision Cancer Medicine Program
Sameek Roychowdhury, MD, PhD
The Ohio State University

4:20 PM - 4:40 PM
Discussion
First Question: Don Coffey

4:40 PM - 4:50 PM	**Microenvironment Targeting of Angiogenesis** **Amado Zurita, MD** The University of Texas MD Anderson Cancer Center	
4:50 PM - 5:10 PM	Discussion First Question: Ralph Buttyan	
6:00 PM - 8:00 PM	Dinner	*Lakeside Ballroom A*

Session V: Plenary Session
Location: *Regency Ballroom DEF*

8:30 PM - 10:00 PM	**Roundtable Discussion:** ***What Should We Be Thinking About?***	**Moderator** Howard Soule, PhD **Participants** Robert S. Kerbel, PhD Martin Gleave, MD James Allison, PhD Owen Witte, MD

SATURDAY, JUNE 22, 2013

	Meet in the Lobby	
6:30 AM - 7:15 AM	"Sunrise Ceremony"	Donald Coffey, PhD
7:15 AM - 8:15 AM	Breakfast	*Beach Barbeque Area*
8:15 AM - 8:30 AM	Move to Session	

Session VI: The AR Binding Domain and Beyond

Location:	*Regency Ballroom DEF*
Session Chair:	**Peter Nelson, MD**

8:30 AM - 8:40 AM	**Introduction to Session** **Peter Nelson, MD** Fred Hutchinson Cancer Research Center
8:40 AM - 8:50 AM	**Targeting Oncogenic Pathways in Prostate Cancer: the SAGA Continues** **Karen Knudsen, PhD** Thomas Jefferson University
8:50 AM - 9:10 AM	Discussion First Question: Scott Tomlins

9:10 AM - 9:20 AM	**SOX9 Regulated Wnt Signaling in TMPRSS2: ERG Fusion Positive and Negative PCa** **Steven Balk, MD, PhD** Beth Israel Deaconess Medical Center	
9:20 AM - 9:40 AM	Discussion First Question: Nicholas Nickols	
9:40 AM - 9:50 AM	**Have We Addressed the Use of the Androgen Receptor by Prostate Cancer?** **Stephen Plymate, MD** University of Washington	
9:50 AM - 10:10 AM	Discussion First Question: Stephen Finn	
10:10 AM - 10:20 AM	**Targeting Transcription Downstream of the Nuclear Receptors** **Nicholas Mitsiades, MD, PhD** Baylor College of Medicine	
10:20 AM - 10:40 AM	Discussion First Question: Matthew Galsky	
10:40 AM - 10:50 AM	**Are We Done With Hormone Therapy?** **Gerhardt Attard, MD, PhD** The Institute of Cancer Research & Royal Marsden Hospital	
10:50 AM - 11:10 AM	Discussion First Question: Tarek Bismar	
11:10 AM - 11:20 AM	Break	*Regency Foyer West*
11:20 AM - 11:30 AM	**Understanding the Neuroendocrine Phenotype as Mechanism of Treatment Resistance** **Himisha Beltran, MD** Weill Cornell Medical College	
11:30 AM - 11:50 AM	Discussion First Question: Rohit Mehra	
11:50 AM – 12:00 PM	**Targeting Aberrant Translational Regulons in Prostate Cancer** **Andrew Hsieh, MD** University of California, San Francisco	
12:00 PM - 12:20 PM	Discussion First Question: Michael Haffner	
12:20 PM - 1:20 PM	Lunch	*Martis Peak ABC*
1:20 PM - 6:00 PM	Afternoon Free	
6:00 PM - 7:30 PM	Dinner	*Castle Peak AB*

Session VII: Plenary Session
Location: *Regency Ballroom DEF*

8:00 PM - 10:00 PM	Brainstorming	**William Nelson, MD, PhD** Johns Hopkins University

SUNDAY, JUNE 23, 2013

6:30 AM - 7:30 AM	Breakfast	*Regency Ballroom DEF*

****Hotel checkout before 12 noon***

Session VIII: What are we forgetting?
Location: *Regency Ballroom DEF*

Session Chair: **Phil Kantoff**

7:30 AM - 7:40 AM	**Genesis of Reactive Stroma and Prostate Cancer Progression** **David Rowley, PhD** Baylor College of Medicine
7:40 AM - 8:00 AM	Discussion First Question: Barbara Lelj-Garolla Di Bard
8:00 AM - 8:10 AM	**Of Men, Mice, and Prostate Cancer** **Colin Collins, PhD** University of British Columbia
8:10 AM - 8:30 AM	Discussion First Question: Mark Rubin
8:30 AM - 8:40 AM	**Targeting Apoptosis and Epigenetics Regulators for New Therapeutics for Prostate Cancer** **Shaomeng Wang, PhD** University of Michigan
8:40 AM - 9:00 AM	Discussion First Question: Jonathan Simons
9:00 AM - 9:10 AM	**Transitioning from Drug to Therapy Development** **Christopher Logothetis, MD** The University of Texas MD Anderson Cancer Center
9:10 AM - 9:30 AM	Discussion First Question: Alexander Wyatt

9:30 AM - 9:40 AM	**Targeting Epigenetic Programs for Prostate Cancer Therapy** **Srinivasan Yegnasubramanian, MD** Johns Hopkins University
9:40 AM - 10:00 AM	Discussion First Question: Amina Zoubeidi
10:00 AM - 10:10 AM	**Bipolar Therapy for CRPC** **John Isaacs, PhD** Johns Hopkins University
10:10 AM - 10:30 AM	Discussion First Question: Lorelei Mucci
10:30 AM - 10:40 AM	**Applications of Functional PET Imaging in Prostate Cancer: Focus on PSMA** **Steve Cho, MD** Johns Hopkins University
10:40 AM - 11:00 AM	Discussion First Question: Stuart Holden
11:00 AM - 11:15 AM	**Meeting Debrief** **Ken Pienta, MD** Johns Hopkins University
11:15 AM	Lunch & Departures

Castration Resistant Prostate Cancer Reveals Intrapatient Similarity and Interpatient Heterogeneity of Therapeutic Kinase Targets

Justin M. Drake, Nicholas A. Graham, John K. Lee, Tanya Stoyanova, Claire M. Faltermeier, Sud Sudha, Bjoern Titz, Jiaoti Huang, Kenneth J. Pienta, Thomas G. Graeber, & Owen N. Witte

ABSTRACT

In prostate cancer multiple metastases from the same patient share similar copy number, mutational status, ETS rearrangements, and methylation patterns supporting their clonal origins. Whether actionable targets such as tyrosine kinases are also similarly expressed and activated in anatomically distinct metastatic lesions of the same patient is not known. We evaluated active kinases using phospho-tyrosine peptide enrichment and quantitative mass spectrometry to identify druggable targets in metastatic castration resistant prostate cancer (CRPC) obtained at rapid autopsy. We identified distinct phospho-peptide patterns in metastatic tissues compared to naive primary prostate tissue and prostate cancer cell line-derived xenografts. Evaluation of metastatic CRPC samples for tyrosine phosphorylation and upstream kinase targets revealed SRC, EGFR, RET, ALK, and MAPK1/3 and other activities while exhibiting intrapatient similarity and interpatient heterogeneity. This suggests that individualized therapy targeting non-mutated kinases with clinical kinase inhibitors may be an effective strategy in the treatment of metastatic CRPC.

In the box below, please indicate your particular activities which justify favorable consideration of you as a participant and contributor to this meeting. This information is important, as it allows the Conference Chair to make an informed decision when reviewing and accepting applications.

I have been in the field of prostate cancer during both Graduate School and as a Postdoctoral Fellow investigating the molecular mechanisms of prostate cancer cell metastases including cancer cell/endothelial cell interactions during metastatic seeding as well as defining activated kinase targets in castration resistant prostate cancer (CRPC). Recently, we found that CRPC patients indeed express activated kinase targets and these patterns of expression are patient-specific. These new findings may help drive new treatment strategies for patients with CRPC. During my Postdoctoral studies in the Lab of Dr. Owen Witte, I have been awarded 2 research grants for my projects in prostate cancer (UCLA Tumor Cell Biology Grant and Department of Defense Prostate Cancer Research Program Grant) and presented my work at 2 Prostate Cancer specific meetings (2012 AACR Advances in Prostate Cancer Research and 2013 Prouts Neck Meeting on Prostate Cancer). Currently, I have 6 first author publications and 3 internal author publications in the field with another first author publication submitted on kinase targets in CRPC. I have a passion for finding new therapies in prostate cancer and would benefit greatly by attending this meeting. Thanks for your consideration.

Hormone-Dependent Cancers

Development and Progression

July 28 - August 2, 2013
Bryant University, Smithfield, RI

Chair: Karen E. Knudsen
Vice Chair: Wayne Tilley

Contributors

The conference also gratefully acknowledges a contribution received in memory of Oliver and Lenore Plymate.

SUNDAY

2:00 pm - 9:00 pm	Arrival and Check-in
6:00 pm	Dinner
7:30 pm - 7:35 pm	Welcome / Introductory Comments by GRC Site Staff
7:35 pm - 9:30 pm	**Hormone Action & Cancer Development** Discussion Leaders: **Karen Knudsen** (Thomas Jefferson University) and **Wayne Tilley** (U. Adelaide)
7:35 pm - 8:00 pm	Keynote Speaker: **Charles Perou** (University of North Carolina at Chapel Hill) "Molecular Genetics and Genomics of Hormone Receptor Positive Breast Cancers for Therapy Selection"

8:00 pm - 8:10 pm	Discussion
8:10 pm - 8:20 pm	**Justin Drake** (University of California, Los Angeles) "Defining kinase targets in castration resistant prostate cancer"
8:20 pm - 8:30 pm	Discussion
8:30 pm - 8:50 pm	**Gail Prins** (University of Illinois at Chicago) "Hormonal Regulation of Prostate Stem and Progenitor Cells"
8:50 pm - 9:00 pm	Discussion
9:00 pm - 9:20 pm	**Geoffrey Greene** (University of Chicago) "Nuclear Receptor Cross Talk and Targeting in Breast Cancer Prevention and Treatment"
9:20 pm - 9:30 pm	Discussion
MONDAY	
7:30 am - 8:30 am	Breakfast
8:30 am	Group Photo
9:00 am - 12:30 pm	**Chromatin Regulation in Cancer Development and Progression: Seq and you will find...**
	Discussion Leaders: **Vasan Yegnasubramanian** (Johns Hopkins University) and **Geoffrey Greene** (University of Chicago)
9:00 am - 9:20 am	**Peter Jones** (University of Southern California) "Cancer Genetics and Epigenetics - Two Sides of the Same Coin?"
9:20 am - 9:30 am	Discussion
9:30 am - 9:50 am	**W. Lee Kraus** (UT Southwestern Medical Center) "Comprehensive Transcriptional Profiling in Breast Cancer Cells"
9:50 am - 10:00 am	Discussion
10:00 am	Coffee Break
10:20 am - 10:40 am	**Carol Lange** (University of Minnesota) "Mechanisms of Altered Progesterone Receptor (PR) Promoter Selection in Breast Cancer Models"
10:40 am - 10:50 am	Discussion
10:50 am - 11:10 am	**Edwin Cheung** (Genome Institute of Singapore) "Global Regulation of Chromatin Structure and Transcription by Nuclear Hormone Receptors"
11:10 am - 11:20 am	Discussion
11:20 am - 11:35 am	**Jindan Yu** (Northwestern University) "Role of FoxA1 in androgen receptor signaling and prostate cancer"
11:35 am - 11:45 am	Discussion
11:45 am - 12:00 pm	**Qianben Wang** (The Ohio State University) "GATA2-dependent transcription programs and prostate cancer initiation"
12:00 pm - 12:10 pm	Discussion
12:10 pm - 12:20 pm	**Laura Cato** (Dana-Farber Cancer Center) "Bag-1L and the androgen receptor"
12:20 pm - 12:30 pm	Discussion

12:30 pm	Lunch
1:30 pm - 4:00 pm	Free Time
4:00 pm - 6:00 pm	Poster Session
6:00 pm	Dinner
7:30 pm - 9:30 pm	**Cross Talk of Hormone Receptor and DNA Damage Response Pathways**
	Discussion Leaders: **Edwin Cheung** (Genome Institute of Singapore) and **Steve Balk** (Harvard Medical School)
7:30 pm - 7:50 pm	**Vasan Yegnasubramanian** (Johns Hopkins University) "The double-edged sword of androgen-induced double strand breaks in prostate cancer"
7:50 pm - 8:00 pm	Discussion
8:00 pm - 8:20 pm	**Felix Feng** (University of Michigan) "Lnc-RNAs, an unexplored frontier in the DNA damage response and cancer phenotypes"
8:20 pm - 8:30 pm	Discussion
8:30 pm - 8:40 pm	**Matthew Scheiwer** (Thomas Jefferson University/Kimmel Cancer Center) "Targeting nuclear receptor-DNA damage crosstalk in cancer therapy"
8:40 pm - 8:50 pm	Discussion
8:50 pm - 9:00 pm	**Dan Gioeli** (University of Virginia) "Checkpoint signaling and AR function in cancer"
9:00 pm - 9:10 pm	Discussion
9:10 pm - 9:20 pm	**Robert Den** (Thomas Jefferson University) "Effectors of the DNA damage and radiotherapy response in cancer"
9:20 pm - 9:30 pm	Discussion
TUESDAY	
7:30 am - 8:30 am	Breakfast
9:00 am - 12:30 pm	**Stem Cells & Cancer Development**
	Discussion Leaders: **Gail Prins** (University of Illinois at Chicago) and **Leslie Gold** (New York University)
9:00 am - 9:20 am	**Geoffrey Lindeman** (University of Melbourne) "Mammary stem cells and breast cancer - taking cues from steroid hormones"
9:20 am - 9:30 am	Discussion
9:30 am - 9:50 am	**Dean Tang** (University of Texas) "Understanding prostate cancer cell heterogeneity and its clinical implications"
9:50 am - 10:00 am	Discussion
10:00 am	Coffee Break
10:20 am - 10:40 am	**Michael Shen** (Columbia University) "Androgen receptor function in prostate epithelial stem cells"
10:40 am - 10:50 am	Discussion
10:50 am - 11:10 am	**Gail Risbridger** (Monash University) "Prostate cancer, stem cells and hormone regulated micro-environment"

11:10 am - 11:20 am	Discussion
11:20 am - 11:35 am	**Scott Cramer** (University of Colorado) "Tak1 and CHD1 intersection with AR signaling"
11:35 am - 11:45 am	Discussion
11:45 am - 12:00 pm	**Suzanne Fuqua** (Baylor College of Medicine) "Role of AR in Hormone Resistance"
12:00 pm - 12:10 pm	Discussion
12:10 pm - 12:20 pm	**Omar Franco** (Vanderbilt) "Role of Androgen Receptor in Prostate Cancer Stroma During Tumor Progression"
12:20 pm - 12:30 pm	Discussion
12:30 pm	Lunch
1:30 pm - 4:00 pm	Free Time
4:00 pm - 6:00 pm	Poster Session
6:00 pm	Dinner
7:30 pm - 9:30 pm	**Microenvironment & Metastasis**
	Discussion Leaders: **Elahe Mostaghel** (University of Washington) and **William Ricke** (University of Wisconsin)
7:30 pm - 7:50 pm	**Kornelia Polyak** (Harvard Medical School) "Therapeutic relevance of epithelial-stromal cell interactions"
7:50 pm - 8:00 pm	Discussion
8:00 pm - 8:20 pm	**Nora Navone** (University of Texas) "Fibroblast Growth Factor axis in the signaling crosstalk between Prostate Cancer cells and Bone"
8:20 pm - 8:30 pm	Discussion
8:30 pm - 8:45 pm	**Theresa Hickey** (University of Adelaide) "Androgen and Estrogen Action in a Complex Cellular Environment"
8:45 pm - 8:55 pm	Discussion
8:55 pm - 9:10 pm	**Alessandro Fatatis** (Drexel University) "New models of bone disease"
9:10 pm - 9:15 pm	Discussion
9:15 pm - 9:25 pm	**Daniel Frigo** (University of Houston) "Functional Role of Autophagy in Prostate Cancer"
9:25 pm - 9:30 pm	Discussion
WEDNESDAY	
7:30 am - 8:30 am	Breakfast
9:00 am - 12:30 pm	**Endocrine Therapy Resistance**
	Discussion Leaders: **Beatrice Knudsen** (Cedars-Sinai Medical Center) and **Carol Lange** (U. Minnesota)
9:00 am - 9:20 am	**Howard Scher** (Memorial Sloan-Kettering) "Understanding And Targeting Resistance To Androgen Receptor Signaling Inhibitors In Castration Resistant Prostate Cancer"

9:20 am - 9:30 am	Discussion
9:30 am - 9:50 am	**Joyce Slingerland** (University of Miami) "How cross talk with Src modifies ligand activated ER transcription coupled proteolysis"
9:50 am - 10:00 am	Discussion
10:00 am	Coffee Break
10:30 am - 10:50 am	**Jason Carroll** (Cancer Research UK) "Understanding estrogen receptor transcription in breast cancer"
10:50 am - 11:00 am	Discussion
11:00 am - 11:20 am	**Leonie Young** (Royal College of Surgeons Ireland) "SRC-1 is a driver of tumour adaptability in endocrine resistant breast cancer"
11:20 am - 11:30 am	Discussion
11:30 am - 11:50 am	**Stephen Plymate** (University of Washington) "Androgen Receptor Splice Variants: A New Set of AR Co-Activators"
11:50 am - 12:00 pm	Discussion
12:00 pm - 12:15 pm	**Ana Aparicio** (MD Anderson) "A Molecular Characterization of the Anaplastic Prostate Carcinomas"
12:15 pm - 12:25 pm	Discussion
12:30 pm	Lunch
1:30 pm - 4:00 pm	Free Time
4:00 pm - 6:00 pm	Poster Session
6:00 pm	Dinner
7:00 pm - 7:30 pm	Business Meeting *Nominations for the next Vice Chair; Fill out Conference Evaluation Forms; Discuss future Site & Scheduling preferences; Election of the next Vice Chair*
7:30 pm - 9:30 pm	**Hormone Action in Cancers Beyond Breast & Prostate** Discussion Leaders: **Michael Shen** (Columbia University) and **Joyce Slingerland** (University of Miami)
7:30 pm - 7:50 pm	**Cheryl Walker** (Texas A&M) "Epigenetic 'Readers, Writers and Erasers' as Targets for Developmental (re)Programming of Tumor Suppressor Gene Penetrance"
7:50 pm - 8:00 pm	Discussion
8:00 pm - 8:20 pm	**Jill Siegfried** (University of Pittsburgh) "Role of Estrogen Receptor in Growth of Lung Cancer"
8:20 pm - 8:30 pm	Discussion
8:30 pm - 8:45 pm	**Elahe Mostaghel** (University of Washington) "Does the Androgen Receptor Play a Role in Mantle Cell Lymphoma"
8:45 pm - 8:55 pm	Discussion
8:55 pm - 9:10 pm	**Xuesen Dong** (University of British Columbia) "Mechanisms of the androgen receptor splicing in prostate cancer cells"
9:10 pm - 9:15 pm	Discussion

9:15 pm - 9:25 pm	**Zhaoyu Li** (Mayo Clinic) "Estrogen action in liver cancer"
9:25 pm - 9:30 pm	Discussion

THURSDAY

7:30 am - 8:30 am	Breakfast
9:00 am - 12:30 pm	**Hormones in Translation**
	Discussion Leaders: **Theresa Hickey** (University of Adelaide) and **Robert Den** (Thomas Jefferson University, Kimmel Cancer Center)
9:00 am - 9:25 am	Keynote Speaker: **Johann deBono** (Royal Marsden Hospital) "Prostate cancer: Just can't get enough (hormones)?"
9:25 am - 9:35 am	Discussion
9:35 am - 9:50 am	**Trevor Penning** (University of Pennsylvania) "Androgen biosynthetic enzymes in castrate resistant prostate cancer"
9:50 am - 10:00 am	Discussion
10:00 am	Coffee Break
10:30 am - 10:50 am	**Cliff Hudis** (Memorial Sloan-Kettering) "The role of the Androgen Receptor in Metastatic Breast Cancer"
10:50 am - 11:00 am	Discussion
11:00 am - 11:20 am	**Steve Balk** (Harvard Medical School) "Androgen Receptor Function in Prostate Cancer Development and Progression"
11:20 am - 11:30 am	Discussion
11:30 am - 11:45 am	**Evan Simpson** (Prince Henryâ€™s Institute of Australia) "Obesity and breast cancer - role of aromatase"
11:45 am - 11:55 am	Discussion
11:55 am - 12:10 pm	**Steffi Oesterreich** (University of Pittsburgh) "Unique Estrogen Signaling in Lobular Breast Cancer: Translational Significance?"
12:10 pm - 12:20 pm	Discussion
12:30 pm	Lunch
1:30 pm - 4:00 pm	Free Time
4:00 pm - 6:00 pm	Poster Session
6:00 pm	Dinner
7:30 pm - 9:30 pm	**The Future of Targeted Therapy**
	Discussion Leaders: **Felix Feng** (University of Michigan) and **Leonie Young** (Royal College of Surgeons Ireland)
7:30 pm - 7:50 pm	**Ganesh Raj** (University of Texas Southwestern) "Targeting the functional social network of steroid receptors"
7:50 pm - 8:00 pm	Discussion
8:00 pm - 8:15 pm	**Jun Luo** (Johns Hopkins University) "Clinical relevance of AR splice variants"
8:15 pm - 8:25 pm	Discussion

8:25 pm - 8:40 pm	**Frank Claessens** (Leuven University) "Genetic variation in the androgen responsiveness of TMPRSS2/T2E fusion"
8:40 pm - 8:50 pm	Discussion
8:50 pm - 9:10 pm	**Robert Clarke** (Georgetown University) "Application of Systems Biology to Explore Targeted Therapies for Endocrine Resistant Breast Cancers"
9:10 pm - 9:20 pm	Discussion
9:20 pm - 9:30 pm	Final Wrap-up and Discussion
FRIDAY	
7:30 am - 8:30 am	Breakfast
9:00 am	Departure

Funding for this conference was made possible (in part) by 1R13CA-177078-01A1 from the National Cancer Institute. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

POSTER LIST

Note: The chairs are planning to provide a detailed poster list handout at the meeting, which will include specific information about which session/day presenters should display their posters, and more. The general alphabetical listing below is provided in the meantime for preview purposes.

Name	Organization	Co-Authors	Poster Title
ADENIJI, ADEGOKE O	UNIVERSITY OF PENNSYLVANIA	Jeffery Winkler and Trevor M. Penning	Pharmacological Characterization of a Bifunctional AKR1C3 Inhibitor and AR Antagonist.
APARICIO, ANA M	MD ANDERSON CANCER CENTER		A Molecular Characterization of the Anaplastic Prostate Carcinomas
ARMSTRONG, CAMERON M	TEXAS A&M UNIVERSITY		Estradiol Suppresses Acute Inflammation in the Colon: a Novel Method for Protecting Against Inflammation-associated Colon Cancer
BARFELD, STEFAN J	CENTRE FOR MOLECULAR MEDICINE NORWAY	Ian G Mills	Exploring the relationship between the AR and c-Myc in prostate cancer
BLESSING, ALICIA M	UNIVERSITY OF HOUSTON	Alicia M. Blessing, Yan Shi, Ganesan Sathya, Emily Y. Lu, Jeffrey T. Chang, Donald P. McDonnell and Daniel E. Frigo	Non-Classical Signaling of the Androgen Receptor Polyproline Domain
BOURGO, RYAN J	UNIVERSITY OF CHICAGO	Geoffrey L. Greene, Ph.D.	Simplified Chromatin Conformation Capture Identifies Plasticity in SIAH2 Gene Architecture
CAO, FEIHUA	AUSTRALIAN NATIONAL UNIVERSITY	Feihua (Lucy) Cao, Kelly M. Morris, Hideki Onagi, Timothy M. Altamore, Allan B. Gamble, Christopher J. Easton	Prohormone-substrate peptide sequence recognition by peptidylglycine a-amidating monooxygenase (PAM) and its reflection in increased glycolate inhibitor potency
CARTER, SARAH L	UNIVERSITY OF ADELAIDE	M. M. Centenera, W. D. Tilley, L.A. Selth and L. M. Butler	Expression profiling of synergistic combinatorial therapy with vorinostat and bicalutamide reveals a key role for NFKBIA in prostate cancer cell death
CATO, LAURA	DANA-FARBER CANCER INSTITUTE	Katja Jehle, Antje Neeb, Andrew C. B. Cato, Myles Brown	Control of androgen receptor action by a novel nuclear receptor binding motif in Bag-1L
CHAN, SIU CHIU	MASONIC CANCER CENTER, UNIVERSITY OF MINNESOTA	Scott M Dehm	Truncated androgen receptor splice variants support constitutive androgen signaling in castration-resistant prostate cancer
CHOI, JAESUNG P	ANZAC RESEARCH INSTITUTE	Choi J, Desai R, Zheng Y, Handelsman DJ, Simanainen U	Androgen actions via androgen receptor promote PTEN inactivation induced uterine growth and pathology

CHU, GINA CHIA-YI	CEDARS-SINAI MEDICAL CENTER	Haiyen E. Zhau, Ruoxiang Wang, Andre Rogatko,Xu Feng, Majd Zayzafoon, Leland W.K. Chung	PREMETASTATIC NICHE INVOLVES RANKL-RANK SIGNALING RECRUITING BYSTANDER CANCER CELLS TO PARTICIPATE CANCER SKELETAL METASTASIS
CHUNG, LELAND W K	CEDARS-SINAI MEDICAL CENTER	Gina C.Y. Chu, Haiyen E. Zhau, Ruoxiang Wang, Andre Rogatko, Xu Feng, Majd Zayzafoon	PREMETASTATIC NICHE INVOLVES RANKL-RANK SIGNALING RECRUITING BYSTANDER CANCER CELLS TO PARTICIPATE CANCER SKELETAL METASTASIS
CINAR, BEKIR	CEDARS-SINAI MEDICAL CENTER AND UCLA	Gamze Kuser Abali and Ahmet Alptekin	Regulation of Androgen Receptor Signaling by YAP in Prostate Cancer Cells
CLAESSENS, FRANK	KU LEUVEN	Clinckemalie L., Helsen C., Spans L., Dubois V., Lerut E., Joniau S. and Claessens F.	Genetic variation in the androgen responsiveness of TMPRSS2/T2E fusion
CLARK, NICOLE C	WASHINGTON STATE UNIVERSITY	Clark NC, Friel AM, Zhang L, McCallum ML, Shioda T, Pru CA, Peluso JJ, Rueda BR, Pru JK	PGRMC1 Mediates Progesterone-Induced Chemoresistance in Breast Cancer Cells and Facilitates Tumor Growth In Vivo
CRAMER, SCOTT D	UNIVERSITY OF COLORADO, ANSCHUTZ MEDICAL CAMPUS	Lindsey Ulkus, Leah Rider. One of these authors will be presenting author.	TBD (Tak1 and CHD1 intersection with AR signaling)
CUKO, EFROSINI Z	UNIVERSITY OF HOUSTON	Daniel E. Frigo	The functional role of pentose phosphate pathway in Prostate Cancer
DE LEEUW, RENEE	THOMAS JEFFERSON UNIVERSITY - KIMMEL CANCER CENTER		Leveraging RB status to define therapy for castrate resistant prostate cancer
DEAN, JEFF	THOMAS JEFFERSON UNIVERSITY	TBD	TBD
DEN, ROBERT B	THOMAS JEFFERSON UNIVERSITY		TBD
DHIMOLEA, EUGEN	DANA FARBER CANCER INSTITUTE	Constantine S. Mitsiades	Non-malignant accessory cells from the metastatic microenvironment induce breast cancer cell resistance to antiestrogens
DRAKE, JUSTIN M	UCLA	Justin M. Drake, Nicholas A. Graham, John K. Lee, Tanya Stoyanova, Claire M. Faltermeier, Sud Sudha, Bjoern Titz, Jiaoti Huang, Kenneth J. Pienta, Thomas G. Graeber, & Owen N. Witte	Castration Resistant Prostate Cancer Reveals Intrapatient Similarity and Interpatient Heterogeneity of Therapeutic Kinase Targets
DU, ZHOU	TONGJI UNIVERSITY		Integrative genomic analyses reveal clinically relevant long noncoding RNAs in human cancer

FAGAN, AILIS	ROYAL COLLEGE OF SURGEONS IRELAND	Jarlath C. Bolger, Jean McBryan, Damian P. McCartan, Christopher Byrne, Eamon Hughes, Fiona T. Bane, Peadar Ó Gaora, Arnold D. Hill, Leonie S. Young	Identification and validation of markers of distant metastases in endocrine resistant breast cancer
FRANCO, OMAR E	VANDERBILT UNIVERSITY	to be submitted at a later date	Role of Androgen Receptor in Prostate Cancer Stroma During Tumor Progression
FRIGO, DANIEL E	UNIVERSITY OF HOUSTON		Functional Role of Autophagy in Prostate Cancer
FUQUA, SUZANNE	BAYLOR COLLEGE OF MEDICINE	Yassine Rechoum, Nancy Weigel	Role of AR in Hormone Resistance
GIOELI, DANIEL G	UNIVERSITY OF VIRGINIA		Checkpoint signaling and androgen receptor function in prostate cancer
GOLD, LESLIE I	NEW YORK UNIVERSITY SCHOOL OF MEDICINE	Savvas C. Pavlides, Kuang-Tzu Huang, Dylan Reid, Stephanie V. Blank, Khushbakhat R. Mittal, Eli Rothenberg,Timothy Cardozo, Bo R Rueda and Leslie I Gold. New York University School of Medicine, NY a	Inhibitors of Skp2 E3ligase block estrogen-induced degradation of nuclear p27kip1 in vitro and in vivo causing inhibition of proliferation: a potential major therapeutic advancement over general prote
GRONER, ANNA C	DANA-FARBER CANCER INSTITUTE	Anna C Groner, Myles Brown	The role of TRIM24 during prostate cancer progression
HAGAN, CHRISTY	UNIVERSITY OF MINNESOTA	Christy R. Hagan and Carol A. Lange	A Common Docking (CD) Domain in Progesterone Receptor-B Links Rapid Signaling Events to STAT5-Dependent Gene Expression Required for Breast Cancer Cell Proliferation
HAYWARD, SIMON W	VANDERBILT UNIVERSITY MEDICAL CENTER		TBD
HILTON, HEIDI N	UNIVERSITY OF SYDNEY	Hilton HN, Santucci N, Silvestri A, Kantimm S, Huschtscha LI, Graham JD and Clarke CL	Changed lineage composition, an event regulated by progesterone, occurs early in breast carcinogenesis
HSU, HANG-KAI	UTHSCSA		TBD
HSU, PEI-YIN	UTHSCSA	Hang-Kai Hsu, Tim Huang	Amplification of Distant Estrogen Response Elements Deregulates Target Genes Associated with Tamoxifen Resistance in Breast Cancer
JIA, LI	WASHINGTON UNIVERSITY SCHOOL OF MEDICINE	Dali Zheng, Katherine N. Weilbaecher, Fanxin Long, Li Jia	Androgen receptor-regulated non-canonical WNT signaling in prostate cancer

KASPER, SUSAN	UNIVERSITY OF CINCINNATI	Premkumar Vummidi Giridhar, Jian Zhou, Nivedita Nivedita, and Ian Papautsky	Separation of circulating tumor cells and cancer stem cells into subtypes using inertial microfluidic devices
KNUDSEN, BEATRICE S	CEDARS-SINAI MEDICAL CENTER	Nishit Mukhopadhyay, Sara Pollan	Drug resistance to c-MET inhibition in prostate cancer
LAM, HUNG-MING	UNIVERSITY OF WASHINGTON	Hung-Ming Lam, Bin Ouyang, Jing Chen, Jiang Wang, Mario Medvedovic, Xiaotun Zhang, Robert L. Vessella, Shuk-Mei Ho	GPR30 as a potential therapeutic target for castration-resistant prostate cancer
LANARI, CLAUDIA	INSTITUTE OF BIOLOGY AND EXPERIMENTAL MEDICINE		TBD
LI, ZHAOYU	MAYO CLINIC	Zhaoyu Li	Estrogen Signaling in Liver Cancer
LIU, GANG	UNIVERSITY OF WASHINGTON		Androgen receptor splice variant ARv567es induces invasive prostatic adenocarcinoma in the mouse
LUO, JUN	JOHNS HOPKINS UNIVERSITY		Clinical relevance of the androgen receptor splice variants
MCBRYAN, JEAN	ROYAL COLLEGE OF SURGEONS IN IRELAND	Jean McBryan, Christopher Byrne, Eamon Hughes, Ailis Fagan, Fiona Bane, Damian McCartan, Jarlath Bolger, Arnold Hill, Leonie Young	Tamoxifen drives metastatic disease progression in endocrine resistant breast cancer
MCILROY, MARIE	ROYAL COLLEGE OF SURGEONS IN IRELAND	Ali A, Bane F , Hao Y , McCartan D, O Gaora P , Hill ADK , Young LS , McIlroy M	Survival benefit conferred by the Androgen receptor is diminished in aromatase inhibitor treated breast cancer.
MOLINOLO, ALFREDO A	NIDCR, NIH		Progesterone receptor isoforms and response to different antiprogestins.
MONTIE , HEATHER	PHILADELPHIA COLLEGE OF OSTEOPATHIC MEDICINE	Diane Merry, Ph.D., Karen Knudsen, Ph.D., Scott Dehm, Ph.D.	Determining the role of androgen receptor acetylation in prostate cancer
MUDRYJ, MARIA	UNIVERSITY OF CALIFORNIA, DAVIS	Alan P. Lombard, Stephen J. Libertini	Low molecular weight androgen receptor forms are expressed in Bladder Cancer cells
MURAKAMI, SHINO	UNIV OF TEXAS SOUTHWESTERN MEDICAL CENTER	Nasun Hah, Anusha Nagari, Charles G. Danko and W. Lee Kraus	Role of Estrogen Receptor-Dependent Enhancer RNAs in Estrogen-Regulated Transcriptional Responses
NARAYANAN, RAMESH	GTX, INC.	Ramesh Narayanan, Sunjoo Ahn, Mitchell S. Steiner, and James T. Dalton	Nonsteroidal, Tissue Selective Androgen Receptor Modulator (SARM) as Targeted Therapy for the Treatment of Androgen Receptor-Positive Breast Cancer

NIKOLOS, FOTIS	UNIVERSITY OF HOUSTON	Fotis Nikolos, Gayani Rajapaksa, Igor Bado, Christoforos Thomas, Jan-Ake Gustafsson	ERß inhibits non-small cell lung cancer cell growth by repressing growth factor signaling
PASCAL, LAURA E	UNIVERSITY OF PITTSBURGH DEPT OF UROLOGY		TBD
PENNING, TREVOR	UNIVERSITY OF PENNSYLVANIA	TBD	TBD
RAJAPAKSA, GAYANI K	UNIVERSITY OF HOUSTON	Gayani Rajapaksa, Fotis Nikolos, Igor Bado, Christoforos Thomas, Jan-Åke Gustafsson	ERß1 increases the sensitivity of breast cancer cells to ER stress by downregulating the IRE-1 pathway of the unfolded protein response
SCHIEWER, MATTHEW J	KIMMEL CANCER CENTER THOMAS JEFFERSON UNIVERSITY	TBD	TBD
SIKORA, MATTHEW J	UNIVERSITY OF PITTSBURGH	Kristine L. Cooper, Amir Bahreini, Soumya Luthra, Uma R. Chandran, Guoying Wang, David J. Dabbs, Alana L. Welm, Steffi Oesterreich	Endocrine response in invasive lobular carcinoma is characterized by unique estrogen-mediated gene expression and de novo tamoxifen resistance
SILVA, ELISABETE F	INSTITUTE FOR THE ENVIRONMENT		TBD
SIMPSON, EVAN R.	PRINCE HENRY'S INSTITUTE		TBD
STOYANOVA, TANYA I	UCLA	Tanya Stoyanova, Aaron R. Cooper, Justin M. Drake, Xian Liu, Andrew J. Armstrong, Kenneth J. Pienta, Hong Zhang, Donald B. Kohn, Jiaoti Huang, Owen N. Witte and Andrew S. Goldstein	Prostate cancer originating in basal cells progresses to adenocarcinoma maintained by luminal-like tumour-propagating cells
TAMAE, DANIEL	UNIVERSITY OF PENNSYLVANIA	Daniel Tamae, Ian A. Blair, Trevor M. Penning	A stable isotope dilution liquid chromatography electrospray ionization selected reaction monitoring mass spectrometry (SID-LC/ESI/SRM/MS) method to analyze androgen metabolism in prostate cancer
TAYLOR, RENEA A	MONASH UNIVERSITY	Renea A. Taylor, Roxanne Toivanen, Mark Frydenberg, Declan Murphy, John Pedersen, Grant Buchanan, David Pook, David M. Berman, Gail P. Risbridger	A Pre-Clinical Xenograft Model Identifies Castration-Tolerant Cancer Repopulating Cells in Localized Prostate Tumours
TELLERIA, CARLOS M	UNIVERSITY OF SOUTH DAKOTA	TBD	TBD

TROTTA, ANDREW P	UNIVERSITY OF ADELAIDE	Eleanor F. Need, Luke A. Selth, Gerhard A. Coetzee, Samarth Chopra, Carole B. Pinnock, Lisa M. Butler, Wayne D. Tilley and Grant Buchanan	The co-chaperone SGTA acts to regulate the androgen receptor and PI3K/AKT activity in prostate cancer cells
ULKUS, LINDSEY	UNIVERSITY OF COLORADO-DENVER	Leah Rider, Cera Nieto, Lina Romero, Scott Cramer	Coordinate loss of CHD1 and TAK1 promotes aggressive prostate cancer with altered androgen signaling
VARESLIJA, DAMIR	ROYAL COLLEGE OF SURGEONS IRELAND	Damir Vareslija, Jean McBryan, Ailis Fagan, Leonie Young	Early adaptive mechanism of estrogen receptor signalling in response to aromatase inhibitor treatment
WANG, QIANBEN	THE OHIO STATE UNIVERSITY		H3K4me1/H3K9me2 mark a novel class of active GATA2 enhancers that direct a GATA2-dependent transcription program associated with prostate cancer initiation
WU, JASON BOYANG	CEDARS-SINAI MEDICAL CENTER	Chen Shao, Xiangyan Li, Peizhen Hu, Yi-Ting Chen, Xiaoliang Dou, Divya Sahu, Wei Li, Hiroshi Harada, Ruoxiang Wang, Haiyen E. Zhau, Leland W.K. Chung	Hypoxia-mediated cancer imaging by a novel class of near-infrared (NIR) heptamethine cyanine dyes
YU, JANE J	BRIGHAM AND WOMEN'S HOSPITAL/HARVARD MEDICAL SCHOO	Yang Sun, Xiaoxiao Gu, Erik Zhang, Tasha Morrison, Chenggang Li, Mi-Ae Park, Shuyan Wang, John Blenis, Victor Gerbaudo, Elizabeth Petri Henske, and Jane Yu	Estradiol enhances glucose metabolism of tuberin-null cells in a metastatic model of lymphangioleiomyomatosis
YU, JINDAN	NORTHWESTERN UNIVERSITY	Hong-Jian Jin, Jonathan C. Zhao, Irene Ogden, Raymond Bergan, Jindan Yu	Role of FoxA1 in androgen receptor signaling and prostate cancer